Risk Assessment for Piscicidal Formulations of Antimycin

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Executive Summary

Antimycin A is a selective piscicide which has not been used in Washington but which may be useful to the Washington Department of Fish and Wildlife (WDFW) as a fisheries management tool in certain circumstances. Antimycin A is particularly useful in circumstances where more rapid degradation, greater selectivity among fish species, or fewer effects on invertebrates is desirable. These desirable features may be offset by its higher price and its limited availability. Antimycin A also is not as effective as rotenone, the piscicide normally used by WDFW, in large bodies of water because of its short persistence and the difficulty of dispersing the product throughout the water column.

Antimycin A is an antibiotic complex produced by and isolated from the actinobacterium, *Streptomyces griseus*. There is currently a single antimycin A product, Fintrol, registered by the U.S. Environmental Protection Agency (USEPA); it is registered only for use as a fish toxicant. Antimycin A acts by inhibiting electron transport in the cellular mitochondria. Unlike rotenone, the effects of antimycin A in fish are not reversible; fish exposed to toxic concentrations will not recover if placed in clean water.

Antimycin A has very recently been reviewed by USEPA in conjunction with its reregistration process. Because of the very specific use as a piscicide and the limited amount used, many of the USEPA data requirements have been waived for antimycin A. The data base on several characteristics is weak and there has been no analytical chemical method to detect and quantify residues in the field until very recently. It has, however, been used as a piscicide since 1964, and there is a substantial data base on fish toxicity, which is augmented by observations from its use. As a result of its assessment, USEPA is proposing some label changes, primarily to reduce the potential exposure to applicators and other persons, but also to ensure more consistent applications such as by requiring certified applicator training and following the use of a "Standard Operating Procedure" manual.

Antimycin A is applied by ground equipment only. If used in Washington, WDFW expects to apply it primarily with drip stations and backpack or mobile sprayers. Potential target sites will be primarily streams and small, shallow ponds. Application rates indicated on the current label suggest, but are not clear, up to 25 parts per billion (ppb) or µg/L, but most applications appear to be at rates of 5-10 ppb. The former rate is non-selective, while the latter rate is selective for salmonids and other species, but not catfish, goldfish, and a few other species. Where transport of antimycin A away from the intended treatment sites is a concern, such as streams, potassium permanganate may be used to deactivate the compound. The proposed label resulting from the USEPA review will require that the maximum rate be clearly stated to be 25 ppb.

Antimycin A has low solubility in water; its vapor pressure is low enough to consider it to be not volatile. It degrades quickly through hydrolysis with half-lives ranging from a few minutes to 15 days. When sediments are used in laboratory studies, degradation was slower than in water alone; reasons are unknown. The pH of the water is very important in persistence, with shorter half-lives occurring at higher pHs; at or above pHs of 8.5-9, it may degrade too quickly to be efficacious. Degradation products are apparently blastmycic acid, antimycin lactone, and antimycic acid. Antimycin A also degrades very rapidly in streams with steep gradients; the basis

is not known, but it has been suggested that high oxygenation may be the cause, or possibly the physical force on the molecules of the antimycin A complex.

Antimycin A adsorbs strongly to sediments, plants, and particulate matter in treated waters. This adsorption, coupled with the reported rapid hydrolysis, indicates that Antimycin will not be available for transport through the sediment/soil column into groundwater; on this basis, USEPA waived requirements for leaching data to directly address groundwater. Due to lack of chemical analytical methods, no groundwater detections are known to have been attempted.

As would be expected from its use as a piscicide, antimycin A is very highly toxic to fish. There are extensive acute fish toxicity data which indicate median lethal (96-hour LC_{50}) values as low as 0.001 µg/L for paddlefish, or in studies used by USEPA in its risk assessment, as low as 0.009 µg/L for coho salmon. Salmonids, in general, are highly sensitive. Catfish are insensitive enough that antimycin A is used to kill other fish in catfish ponds. Other species are intermediate. Fish are more sensitive at lower pHs and higher temperatures. The pH factor is most likely related to degradation of antimycin, but the data are too limited to determine if the temperature aspect is due to degradation or the physiological action of antimycin A. No chronic toxicity data for fish could be found.

Very limited laboratory data indicate that toxicity to aquatic invertebrates can be quite high; the most sensitive species tested, an amphipod, had an LC_{50} of 0.008 µg/L. Other invertebrates, including amphipods, were far less sensitive. They had LC_{50} values ranging from 0.146 µg/L for a midge to 15 µg/L or higher for other species of arthropods, mollusks, and platyhelminths. These values indicate very high toxicity, but when compared with application rates and fish toxicity, the effects from actual use would not be as pronounced as would be indicated by LC_{50} values alone. Simulated field studies indicated that a variety of insect species were unharmed at 20 µg/L and field observations under actual use conditions indicate that effects are minimal or none at application rates of 5-10 µg/L in several studies. At higher application rates ranging from 40-100 ppb, field effects on invertebrates were obvious but the duration of such effects was generally short, with recovery of populations in several months to a year.

Gilled stages of amphibians are moderately sensitive to antimycin A, although data are limited. LC_{50} values have been determined to be above 10 µg/L for the most sensitive tested species, *Ascaphus truei*. Other amphibians were less sensitive, but much of the data were reported as LC_0 and LC_{100} values and are not comparable. Observations following antimycin A treatments have noted dead amphibians, two salamanders, only once.

In simulated field tests and field observations after treatments, no effects were noted on aquatic macrophytes or phytoplankton.

Among terrestrial organisms, for which exposure is expected to be minimal, technical antimycin A has high to very high toxicity, with birds having LD_{50}s as low as 2.9 mg/Kg for mallard ducks. There are no chronic toxicity data for birds. Mammalian acute data are available only for Fintrol, 20% a.i. antimycin A, and show rat LD_{50}s of 286 mg/Kg for males and 361 mg/Kg for females. The 90-day rat study had a no-observed-adverse-effect-level of 0.5 mg/Kg/day, based on diarrhea, which probably resulted from antibiotic effects on intestinal flora. No terrestrial phytotoxicity data are known.

Indirect effects may occur for organisms that rely on fish or aquatic invertebrates, resulting from loss of a food supply. Typically, terrestrial organisms can find other locations or types of food sources. For aquatic organisms, such effects will typically be brief. It is important to the

purposes of using piscicides that the food sources for restocked fish be available. With the rapid degradation of antimycin A and its limited effects on invertebrates, these indirect effects are most likely transient, and are less likely to occur with the proposed maximum application rate of 25 µg/L.

Effects on threatened and endangered (T&E) species are not expected. While T&E fish are quite susceptible, antimycin A would not be used where they occur except in conjunction with permits from the National Marine Fisheries Service and the Fish and Wildlife Service. Exposure to terrestrial T&E species is highly unlikely. Aquatic T&E plants would not be sensitive to antimycin A.

The toxicity data base with respect to humans is very incomplete. The rat LD_{50} for combined sexes, 316 mg/Kg for the 23% Fintrol product, was found to warrant the highest toxicity classification, with inhalation also warranting a concern, but only for the technical grade. Eye irritation is enough of a factor to prohibit the use of contact lenses by applicators in the proposed labeling.

Because of the very limited and specific use of antimycin A, USEPA took the approach of not requiring additional data to address health effects. Rather, the proposed labeling attempts to preclude any human exposure, based upon the nature of the use and the rapid degradation. Consequently, stringent limitations are proposed to ensure that exposure, and thus effects, will not occur to either applicators or to others who might be exposed to treated water. USEPA invited the submission of additional data that might be used to remove some of these exposure limitations, but did not require such data.

Although there are some uncertainties, the available data and the experience from using antimycin A for fish control purposes indicate that it can be used safely. Laboratory toxicity data for invertebrates and other non-target taxa appear, at first glance, to be high, but there is a wide margin between toxicity to fish and toxicity to all but very few other species. Beyond the intended target fish, some direct effects may occur on certain aquatic invertebrates at the high end (25 ppb) of the proposed application rates. Transient, limited effects could occur at more typical rates of 5-10 ppb, but the available data indicate that these would be neither substantial nor significant. At labeled application rates, the limited data appear to indicate negligible concern for human health effects. However, with the proposed measures to preclude exposure, there will be no effects on humans from labeled use of antimycin A.

1. Introduction

The Washington State Department of Fish and Wildlife (WDFW) is responsible for management activities relating to recreational fishing within the State of Washington. Maintaining a high quality fishery sometimes requires intervention on the part of fisheries managers to enhance habitat for threatened, endangered, and other desirable species, to remove introduced fish that may compete with or prey upon native fish species, to control diseases, to sample fish populations, or for other reasons. Piscicides have long been used in Washington to achieve these goals.

Antimycin A is an effective and selective piscicide, but has not been used in Washington. It has a small market in the U. S. Less than 200 pounds are used yearly for fisheries management, and a

similar additional amount is used in catfish farming. Rotenone has been the only piscicide used in Washington in the last several decades. But antimycin A has several features, especially its selectivity, that would make it an advantageous tool to have available to fisheries managers.

1.1 Background

WDFW has been using piscicides in its fisheries management program since 1940 (WDFW, 2002). Although others chemicals were used long ago, rotenone is the only piscicide used in Washington over the last several decades. Antimycin A was discovered in 1945 (USEPA, 2006a). It was patented as a piscicide in 1964. Like rotenone, antimycin A was already a registered pesticide when EPA was formed in 1971 and the registration was transferred to EPA. In 1993, antimycin A was subjected to a re-registration review. Certain changes in labeling and some minimal data requirements ensued. Antimycin A is again undergoing an assessment for reregistration; a Reregistration Eligibility Decision document has been completed and was signed on May 16, 2007. It was posted to the Antimycin A docket on June 13, 2007 and a public comment period was opened until August 13, 2007.

1.2 Objectives

The objective of this analysis is to provide an up-to-date ecological and human health assessment of antimycin A for use by WDFW. In 2005, WDFW completed a State Environmental Protection Act review for the use of antimycin A in its lake and stream rehabilitation program. It is expected that this risk analysis will be used to support an application by WDFW for the renewal of a National Pollution (NPDES) permit for their piscicidal use of antimycin A for fish management purposes should WDFW choose to use it.[1]

1.3 Methods

This assessment draws heavily upon documents developed by the U. S. Environmental Protection Agency (EPA) as part of EPA's reregistration process for pesticides. A "Reregistration Eligibility Document" (RED) for antimycin A was signed May 16, 2007 (USEPA, 2007). Separate documents containing much more data and information to support the RED have been developed and can be found on EPA's antimycin A docket at http://www.regulations.gov/fdmspublic/component/main, (search for docket # EPA-HQ-OPP-2006-1002) was initially visited March 20, 2007 and again on June 15, 2007. Additional fish toxicity data were obtained from EPA's ECOTOX data base (http://cfpub.epa.gov/ecotox/, accessed April and May 2007), and other literature sources. Because antimycin A is a piscicide, there are several older studies addressing an array of fish toxicity data. Comparative 96-hour toxicity data have been captured from these studies in the ECOTOX data base, but there are additional data in these publications on no-effect-levels and 100%-effect levels, which would be used in looking at selective efficacy. As efficacy is not the goal of this health and environmental assessment, these efficacy data are not included here. Beyond the fish toxicity data, there is relatively little information available for antimycin A. It has never been registered for any pesticidal uses other than as a piscicide, and many of the standard studies, including most fate and transport data associated with terrestrial uses, have been waived. Because antimycin A is a useful niche chemical, and because of the small market and the expense of studies to support

[1] NPDES permits are no longer required for pesticides labeled for aquatic use. (*Federal Register* 71(227): 68483-68492, November 27, 2006.). However, the Washington Department of Ecology has determined to continue its issuance of a combined NPDES/State Waste Discharge Individual Permit for the use of aquatic-use pesticides in the State of Washington.

registration, no additional data are being required by EPA in conjunction with reregistration. As with rotenone, much of the limited information on antimycin A is from the older literature and could only be obtained through its inclusions in more recent summaries in the time frame available for this assessment.

2. Problem Formulation

An analysis of the potential piscicidal use of antimycin A in Washington state first requires a problem formulation such as that described in EPA's Framework for Ecological Risk Assessment (USEPA, 1992), and updated in the Guidelines for Ecological Risk Assessment (USEPA 1998). A problem formulation describes the nature of the stressor agent, antimycin A in this case, considerations of the intentional and unintentional receptors of that stressor, and the effects of the stressor on those receptors. This section defines the scope of the assessment in terms of the stressor, the receptors, and the methods and models used to quantify and characterize the effects of the stressor on the receptors.

The purpose of this assessment is to provide updated information on antimycin A for WDFW to consider in making a decision whether to use antimycin A in the fisheries rehabilitation program, and if it is determined that antimycin A should be used, then a further purpose is to provide an assessment to inform WDFW and to support WDFW's application to the Washington Department of Ecology for the renewal of their NPDES permit for fish management.

Antimycin A is a pesticide currently registered for use as a piscicide. The reigning paradigm for pesticides is that, for each type of receptor organism, there will be doses or concentrations of that pesticide that will affect those organisms and lower doses or concentrations that will not affect those organisms. Theoretically, there is a continuum, or dose-response, where increasing doses will result in increasing effects ranging from "no effect" to 100% effect on various types of receptors. This dose-response concept is well accepted in toxicology for the greatest part. However, there are debates regarding the theory as one approaches either the no effect dose or concentration and the 100% effect dose or concentration. For example, Calabrese and Baldwin (2003) have long maintained that some low doses of what are normally considered toxins in human toxicology studies may actually be beneficial, and Chapman (2001) has applied the same concepts to ecological toxicology and risk assessment. This phenomenon is called hormesis.

A typical risk assessment includes the nature and quantity of exposure of receptors to the stressor, the toxicology of the stressor to the various receptors, or surrogate organisms for those receptors, and a characterization of the effects. In the case of rotenone used as a piscicide, there were two areas of special concern to the Washington Department of Fisheries and Wildlife. The first area related to the potential that rotenone may cause Parkinson's disease, and the second area involved the potential for rotenone used in lakes to move through fractured basaltic substrates into groundwater that may supply drinking water wells. This antimycin A assessment will provide an emphasis on the groundwater concern because the conceptual concern for rotenone would apply to any piscicide used in Washington. However, there is no evidence of any relationship between antimycin A and Parkinson's disease or other neurological toxicity, and this latter issue will not be addressed.

The U.S. EPA signed off on a Reregistration Eligibility Decision (RED) document for antimycin A on May 16, 2007, and posted it on its website on June 13, 2007. There are no further data requirements for antimycin A (Lance Wormell, SRRD/OPP, telephone communication, May 21, 2007), but there are a number of risk mitigations and labeling requirements to achieve these

mitigations (see section 3.4 and Appendix 1). The labeling requirements included in the RED will be subject to a public comment period after the RED becomes publicly available.

2.1 Objectives of use of piscicides by WDFW

Among their responsibilities, the Washington Department of Fish and Wildlife is charged with maintaining a viable recreational fishery in waters under their jurisdiction. For many such waters, the introduction of non-native species has occurred widely, leading to impaired fisheries as a result of competition, predation, or alteration of key parts of ecosystems. There have also been significant perturbations that have resulted in changed environmental conditions that may affect native fish populations. To fulfill their legislative mandate regarding fisheries, WDFW may use piscicides to control fish whose populations may have become imbalanced with respect to demographics or species composition, or may have otherwise impaired the fishery. WDFW may also use antimycin A to aid in the recovery of threatened and endangered (T&E) fish species in Washington.

Like rotenone, antimycin A may be used when all fish in a body of water are to be eliminated, with subsequent stocking of desired fish to rehabilitate the fishery. More frequently, antimycin A is used as a selective piscicide on certain kinds of fish. For example, because of its relatively low toxicity to catfish, the majority of the antimycin A use in the U. S. is in catfish farming in the southeastern U.S (EPA, 2006b) to control other fish in catfish aquaculture ponds; this use in catfish farming is not part of this analysis.

The most frequent current, selective use of antimycin A in fisheries management situations has been to eliminate introduced salmonid species so that native species of concern can be reintroduced and not be subject to competition, predation, or hybridization with the introduced salmonids. Its selectivity and rapid degradation allow fisheries managers to use it in conjunction with management of other kinds of species. At the labeled application rates, antimycin A has limited effects on macroinvertebrate fauna, thus allowing for a more rapid repopulation of fish that depend heavily on a macroinvertebrate food supply.

2.2 Types of sites where piscicides may be used

According to the 2002 WDFW revised plan for using rotenone (WDFW, 2002), most fish rehabilitation has occurred in lakes, ponds, and reservoirs, although a few streams in eastern Washington have been treated to enhance resident trout. There is no history of antimycin A use in Washington. In the 2005 Environmental Checklist prepared for the State Environmental Protection Act review and approval of antimycin in the State, WDFW stated that this product may be used statewide, in lakes and streams where the need has been identified to remove exotic or undesirable fish species for rehabilitating and recovering native fish populations or other native aquatic communities. The treatments would be conducted to eliminate non-native undesirable fish species, to the benefit of native fish, species of concern, or desirable species and stocks. Treatments may occur statewide to remove deleterious species that have the potential to adversely impact native species in the aquatic system.

According to the EPA, antimycin A is generally used "to repopulate native, threatened, or endangered trout species in streams by eliminating nonnative fish species, particularly in high-altitude alpine lakes and streams because it is effective in cold alpine waters and where pH is low. In addition, this piscicide may be used at low concentrations, which makes it easy to transport to isolated or hard to reach mountainous streams. Rotenone is most often used in standing water, such as large lakes and reservoirs, and that it was often applied to maintain sport fisheries, sample fish populations, and in rearing ponds" (USEPA 2006b). However, rotenone has also been used

in mountain streams to aid in the restoration of threatened and endangered trout species (e.g., Finlayson, et al., 2001).

Conversely, antimycin is not as effective as rotenone in large bodies of standing water primarily because of its short persistence and the difficulty of dispersing the product throughout the water column. The Fintrol Concentrate label states that it is designed for use in running water, streams, and shallow waters. Prior to their cancellation in 1986, Fintrol 5 (1% a.i.) and Fintrol 15 (5% a.i.) were also registered as piscicides. Older labels, when all three Fintrol products were available, recommended the use of Fintrol Concentrate (the currently registered formulation) for shallow waters, Fintrol 5 for waters up to 5 feet deep, and Fintrol 15 for waters 15-20 feet deep. The old Fintrol Concentrate label also states that it "releases toxicant to a depth of 2 to 3 feet."

Pfeifer et al (2001), in their review of Washington's high lakes management program stated that the greatest value of antimycin is because it is degraded extremely rapidly when it comes in contact with sunlight and strong oxygenation. As a result, it does not need to be detoxified before reaching downstream fish populations when it is used in high altitude streams and lakes having outlets with steep stream gradients. Finlayson et al. (2002) stated that most (76%) respondents to a questionnaire on antimycin use had neutralized treated waters with $KMnO_4$. WDFW expects that neutralization of treated water in stream rehabilitations may be a management option. Both Finlayson et al. (2002) and Pfeifer et al. (2001) noted the advantage in that antimycin A does not have as serious an impact on aquatic invertebrates as rotenone.

2.3 Nature of antimycin A as a stressor

Antimycin A is an antibiotic produced by and isolated from the actinobacterium, *Streptomyces griseus*. It is actually a complex of eight related compounds, 4 major homologues and 4 minor ones (USEPA, 2006a). It was first discovered in 1945, and patented as a fish toxicant in 1964. It has been registered as a piscicide in the U. S. and Canada since 1966 (Lennon et al., 1970). Antimycin A acts by inhibiting electron transport between cytochrome *b* and cytochrome *c* in Complex III in the cellular mitochondria (USFWS, 2007). Unlike rotenone, the effects of antimycin A in fish are not reversible. Once fish are exposed to effective doses, they will not recover if placed in clean water (Lennon et al., 1970).

Antimycin A is relatively short-lived in the environment. This characteristic provides an advantage not only in the duration of toxicity, but also because restocking can be done more quickly and deactivation is needed in fewer situations. The short life can be a disadvantage in that a second application may be needed. If it is necessary to reduce antimycin A concentrations faster than would occur naturally, potassium permanganate can be used to detoxify the compound. The new proposed labeling would require deactivation by potassium permanganate for any lotic water treatments prior to the treated water leaving the target area.

There is limited use of antimycin A in the United States. EPA determined that less than 200 pounds of antimycin were used in 1998 (USEPA, 2006b); estimates were not provided for other years. However, the American Fisheries Society estimated that a total of 1138 Kg of antimycin A were used by state and federal fisheries resource managers between 1991 and 2001 (USEPA 2006a). It has been noted that antimycin A product availability is limited (Finlayson, et al., 2000).

Antimycin A is often thought of as a costly pesticide. However, in EPA's alternatives analysis, it was determined that the cost, based upon 1998 data, was comparable to rotenone on the basis of treating a specific amount of water. The cost of antimycin A was estimated to be about $47/acre-

foot of water; no distinction was made between selective control application rates and the higher rates allowed on the label. Rotenone cost was found to be $2-13 per acre-foot at unspecified "low rates" and $45-137 at high rates (USEPA, 2006b). Antimycin A may be more costly per unit volume of product, but it requires much lower application rates than rotenone.

It is notable that there has been no good analytical method for antimycin A. Thus, bioassays have been used to determine whether antimycin A exists in concentrations toxic to fish. Kenneke (2006, attachment to USEPA, 2006a) had to develop an analytical method for each of the eight components in antimycin A to investigate its hydrolysis. As a result of Kenneke's work, there is now an analytical method that can quantify antimycin A concentrations down to 0.015 parts per billion (ppb or μg/L) of active ingredient (a.i.).

There has been some confusion in the literature regarding the percentage of active ingredient in Fintrol Concentrate. The current label states that the weight/weight percentage is 23% a.i. The same label refers to it being a 20% solution; this presumably is on a weight/volume basis, but is not entirely clear. However, the older labels refer to it being a 10% solution, which would be the case if the concentrate and equivolume diluent were mixed together. This may account for the tests indicating 23% a.i., 20% a.i., and 10% a.i, were used when they apparently all involved "Fintrol Concentrate."

2.4 Ecological receptors that may be exposed to antimycin A use

Ecological receptors that would be exposed to the use of antimycin A are primarily aquatic organisms of all taxa, along with human applicators. While potential exposure of terrestrial organisms as a result of spray drift cannot be completely ruled out, it is highly unlikely. Antimycin A is generally introduced directly into or immediately over the water. Applications made at ground level, such as from a boat, backpack sprayer, or by drip stations typically have limited amounts of drift.

The aquatic phytotoxicity data are limited, but there is enough information to conclude that effects on aquatic plants are unlikely. There is no reason to think that terrestrial plants would be sensitive, even in the unlikely event that they would be exposed. Similarly, exposure of terrestrial animals is expected to be minimal, and given the low application rates, there is no concern for risks to terrestrial vertebrates, even those that might feed on fish killed by antimycin A (EPA, 2006a). Therefore, the focus of the risk among ecological receptors is primarily oriented towards aquatic animals. However, the low likelihood that terrestrial organisms feeding upon aquatic animals may be indirectly affected by a loss of their food base warrants some discussion.

Aquatic animals and ecosystems will be exposed to antimycin A when it is used as a piscicide. Fish are the intended receptors, but exposure of all types of aquatic organisms is unavoidable from this use. The antimycin A label specifies the amount of exposure in the water column that would occur in accordance with label directions for using antimycin A; 25 ppb is the maximum amount of exposure specified on labels, but often a lower rate is used. Thus, species in the water column, such as fish, amphibians, aquatic arthropods, mollusks, zooplankton, phytoplankton, and aquatic macrophytes could be exposed to a maximum of this 25 ppb concentration. Fish, mollusks, amphibian neonates and larvae, and aquatic invertebrates may become exposed to rotenone directly via uptake through gill tissues. Adult amphibians may become exposed through dermal respiration. EPA maintains that the 25 ppb maximum is not rigid on the label (USEPA, 2006a), but the new proposed labeling will clarify it as a maximum (USEPA, 2007).

Based upon its physical-chemical properties, antimycin A should partition to sediments or particulate matter, including plants, in the water column. However, the lack of a good analytical method for detecting low level antimycin A residues in environmental samples has resulted in a dearth of quantitative residue data.

Antimycin A may be ingested in drinking water or as residues in aquatic food sources for certain types of birds and mammals. Birds, mammals, and reptiles could be exposed through dermal contact while in treated waters. For many such species, such exposure would be transient. It is assumed that species exposed frequently, such as piscivorous birds, ducks, muskrats, garter snakes, and others, would be most at risk from the use of antimycin A and that the risk from transient exposure would be relatively insignificant.

2.5 Considerations of human exposure

Humans may be exposed to antimycin A in several ways. The highest potential exposure would be from the preparation and application of antimycin A. Dermal and inhalation exposure would be the primary routes of exposure for applicators. The current antimycin A label indicates that swimming is not allowed until a bioassay of sensitive fish shows they survive for 48 hours in the treated waters. The same requirement applies to use of treated water for drinking or irrigation. Thus, there would be no human exposure to antimycin A, other than from the applications, for a minimum of 48 hours after application. The current Fintrol label also states that fish killed by antimycin A "should" not be consumed by humans or livestock. The proposed label changes (section 3.4 and Appendix 1) prohibit use of treated water for drinking, irrigation or swimming until measured antimycin A residues drop below the 0.015 µg/L detection limits. In addition, the "should not" term on fish consumption has been proposed to be replaced by a prohibition.

3. Label Description and History

3.1 Antimycin-A registered product

There is a single end-use product of Antimycin A, Fintrol Concentrate Fish Toxicant Kit. The "kit" is actually composed of two parts, the Fintrol Concentrate, 23% (w/w) antimycin A, and the Fintrol diluent with no pesticide active ingredients. Each of the two components is 8 fluid ounces. This product has been given Restricted Use Classification by EPA, which means that it may be applied only by certified applicators who have been trained.

Prior to their cancellation in 1986, two other formulations were registered. These are Fintrol 5 (1% a.i.) and Fintrol 15 (5% a.i.). They were apparently formulated by coating sand grains with antimycin A, although the formulation details of even cancelled pesticides are considered Confidential Business Information (CBI) and are not available. It is not known why these products were cancelled.

3.2 Application methods and rates

Application methods are intended to be by ground equipment only. The label states that aerial application "is NOT recommended." As stated on the current label, this is only guidance and would not be considered an enforceable requirement. The proposed label changes do not address aerial application; however, EPA's assessment is based upon only ground applications.

The label indicates how much of the product is needed per acre-foot of water to achieve concentrations of 1-10 ppb; presumably higher concentrations up to the 25 ppb maximum on the label can be calculated by the applicator. The label does provide information on how to calculate

the amount of acre-feet to be treated. Once the needed amount has been determined, the concentrate in the fish toxicant kit is mixed with the accompanying diluent. The mixture is then diluted with at least 5 gallons of water. Once diluted with water, all of the pesticide must be applied within an 8-hour period.

The label indicates that, after mixing and diluting with water, Fintrol can be applied in lentic waters by the boat bailer method or by spray equipment. Spray methods are indicated as useful to a depth of one foot. WDFW expects that they will use antimycin A with drip stations and backpack/mobile sprayers as the primary means of application. The drip station procedures are similar to those described by Finlayson, et al., 2000 for rotenone. Pinpoint applications by backpack sprayer can be made in shoal areas and around small isolated ponds.

For stream applications, the label indicates that Fintrol is most often applied through drip stations established to meter the toxicant at the precalculated rates. Placement of stream stations depends upon the flow rates of the waters to which it is applied.

The label also recommends that all applications of Fintrol be made at daybreak or as soon as there is enough light to work by.

3.3 Efficacy and selectivity of the antimycin-A product

Antimycin A is promoted on the Fintrol label as a selective pesticide at lower rates or for complete fish kills at higher rates. According to this label, catfish, short nose gar, bowfin and goldfish are not very sensitive to concentrations of antimycin A when it is used selectively at concentrations of 5-10 ppb. When used at higher concentrations of 15-25 ppb, then even these tolerant species would be killed. On this basis, the lower concentrations can be used to eliminate unwanted fish in catfish farming without much, if any, effect on the catfish.

The same kind of selectivity makes antimycin A useful for eliminating non-native trout in waters where they have been introduced, as a prelude to reintroductions of native fish, often threatened or endangered trout. Obviously, other sensitive fish would be killed along with the non-native trout, but tolerant fish would survive and some individuals of species with intermediate sensitivity would also survive. As a result, there would be less perturbation of the treated water than if all or most fish were killed. The toxicity data base for fish is moderate (see section 6.3.4 below), but most fish species that might occur in waters to be rehabilitated would not have been tested. So it is unclear the array of fish species that might be affected by a selective use of antimycin A. Bioassays may be used for determining other species that are sensitive or tolerant if these species are important to be retained in treated water. While fingerling bluegill sunfish are considered to be a sensitive species on the label, centrarchids are generally less sensitive than salmonids, and could be left if a low concentration treatment was used for trout, except that water temperatures frequently mean that the two groups do not commonly co-occur in streams (especially) that are treated.

In addition to leaving more tolerant fish in treated waters, the use of antimycin A would have less effect on macroinvertebrates. Again, there is some selectivity, with the most sensitive invertebrates being approximately as sensitive as the most sensitive fish. But the laboratory toxicity data and the observations made in the field following selective treatments indicate that antimycin A has much less effect than the other available piscicide, rotenone (USEPA, 2006a, Pfeifer et al., 2001).

3.4 Risk mitigations and expected label changes

The antimycin A RED (EPA, 2007) specifies several risk mitigations and a number of label changes necessary for the end-use antimycin A products to be reregistered. The risk mitigations in Table APP-1 are the steps that need to be taken to address risk concerns, and Table APP-2 indicates the specific labeling requirements intended to achieve the risk mitigations. These are reproduced completely in Appendix 1, except for stating where on the label the statements are to be placed. While the RED is technically "final," there will be a public comment period for 60 days, and there is a potential for some of these statements to be changed.

Many of these requirements are pre-emptive based on a lack of data. The RED indicates that if valid data are provided regarding various features of toxicity, exposure, and persistence, the requirements may be reduced, depending upon the data provided. There are, however, no data requirements for antimycin A if all of these risk mitigations appear on the label.

One expected label requirement was not included. The current label states that "Application from an airplane is NOT recommended" (emphasis in original). This recommendation is not the same as a prohibition. WDFW does not expect to treat waters with antimycin via aerial application.

Key features of the proposed labeling include:

- A Standard Operating Procedure (SOP) manual, approved by EPA, must accompany the product and is considered labeling and is therefore mandatory. An SOP manual is being developed by the National Park Service (Lance Wormell, SRRD/EPA, email communication, May 22, 2007)
- Antimycin A end-use formulations will continue to be classified for Restricted Use requiring that applications be made by trained certified applicators. The basis for Restricted Use classification is high acute toxicity to fish and other aquatic organisms and the need for specialized training in its use. There are minor changes specified for the wording on the label regarding classification. In addition, the label will specify that the Certified Applicator will be responsible for notifying drinking water authorities, placarding the area to prohibit entry into the area for 7 days, and prohibiting consumption of dead fish. See Appendix 1 for additional details.
- The Personal Protective Equipment (PPE) requirements have been strengthened. Previous labels included only goggles and gloves. Long-sleeved shirts, long pants, shoes and socks are now also required for all persons handling antimycin A. Those persons handling the concentrate and applicators applying antimycin A with handheld equipment or nozzles must also wear a dust/mist respirator. Persons entering treated water within 7 days must wear coveralls. See Appendix 1 for additional details.
- The label must specifically prohibit application to estuarine or marine waters.
- Use of treated water for drinking, irrigation, or swimming is only allowed after chemical analyses indicate that antimycin A concentrations are below the 0.015 µg/L level of detection.
- The label must specifically prohibit applications at more than 25 µg/L; current labels imply this but are not specific enough.
- Water leaving the treatment area must be deactivated with potassium permanganate to prevent exposure beyond the defined treatment area. Instructions are in the Antimycin A SOP Manual.

4. Chemical Characteristics

The physical/chemical data in the following sections are those required by USEPA when a product is registered for use in the US as a pesticide. These characteristics assist in the basic understanding of the molecule and are later used in predicting environmental behavior or are considered when higher tiered studies are designed or requested. Pure active ingredient or technical grade active ingredient refers to the active compound(s), which cause the desired biological effect when applied to a target system. The technical grade active ingredient is typically formulated into end-use products, also known as formulated products. The end-use products consist of a known percentage active ingredient plus a solvent or solid carrier and may include surface active components to aid in dissolution, emulsification, suspension, *etc.*, of the active ingredient. Technical products such as Antimycin A are rarely the desired form in the end-use product. One method used to produce a useful end-use product is to combine the technical grade active ingredient with solvents or diluents and surface active ingredients to assist their distribution in the aquatic environment. These products are typically either aqueous solutions which easily disperse into water, or emulsifiable concentrates which use the surfactants to allow the active ingredient to mix easily with water and therefore disperse in the treated water body.

4.1 Composition of the single antimycin A end-use product

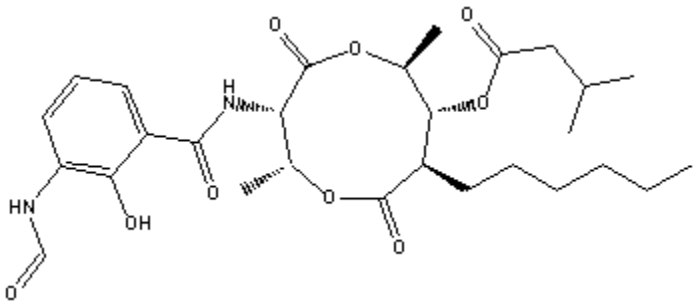
Antimycin A is a mixture of antimycins obtained by extraction and isolation from cultures of *Streptomyces griseus* and is the active component in products used as piscicides in lentic (standing) and lotic (flowing) water to eliminate fish. Antimycin A is a naturally occurring bacterial product extract that exhibits its pesticidal action by uncoupling oxidative phosphorylation in the cell mitochondria by blocking electron transport at complex III. The molecule binds at site Qi and results in the formation of large quantities of Superoxide, a toxic free radical. (Finlayson, 2000; Ware, 2000)

Antimycin A is formulated as a kit in that it consists of two containers, a concentrate containing the active ingredient and a diluent where the two are mixed immediately before application. The product is available under the trade name Fintrol Fish Toxicant Kit Fintrol Toxicant (contains 23% Antimycin A Solution) (EPA Reg. No. 39096-2). This is the only currently registered product containing Antimycin A.

4.1.1 Active ingredients

Antimycin A is a relatively complex molecule containing carbon, hydrogen, nitrogen and oxygen. There is no sulfur, halide, metal or other element that could contribute to persistent or exotic degradates/metabolites.

Common name: Antimycin A
CAS Registry No.: 1398-94-0
Chemical name: Mixture of antimycins
IUPAC name: 3-methylbutanoic acid 3[[3-(formylamino)-2-hydroxylbenzoyl]amino]-8-hexyl-2,6-dimethyl-4,9-dioxo-1,5-dioxonan-7-yl ester
Empirical formula: $C_{28}H_{40}N_2O_9$
Molecular weight: 548.63
Structure: (dotted lines indicate stereo chemistry of Hydrogen groups)



4.1.2 Impurities

Information on impurities, other than the associated active ingredient, is part of the Confidential Statement of Formulation, and because it is Confidential Business Information, it is not available.

There are no known impurities identified by the manufacturers or the US EPA which are known to be of toxicological or environmental concern. The US EPA has established guidelines that require that impurities of concern, such as N-nitrosoamines and chlorinated dioxins and furans must be disclosed. No such compounds are known to be present in the Antimycin A products.

Intentionally added inert or "other" ingredients in the Antimycin A formulation include: soy lipids and acetone in the concentrate and diethyl phthalate, nonoxynol-9 and acetone in the diluent.

The USEPA has established a category listing system for the "other" (inert) compounds used in pesticide formulations. The lists are designated 1, 2, 3, 4a and 4b. Compounds are assigned to the various lists according to their toxicological concern and to the extent their safety has been reviewed by the Agency. In the case of each list, if USEPA determines that a compound is no longer used in any pesticide formulation, it will be removed from the list.

List 1 contains eight compounds, which, due to their toxicological profile, require special labeling if used in a pesticide formulation. These compounds are generally not used in pesticidal formulations any longer. There are no List 1 compounds in the Antimycin A formulations used in the State of Washington.

List 2 compounds are those for which USEPA has not yet determined a full profile but is reviewing existing information. At the completion of their evaluation, it is expected that the compounds still in use in pesticide formulations will be moved to List 1 or to List 4. There is one List 2 compound in the Antimycin A formulation used in the State of Washington – diethyl phthalate, a surfactant contained in the diluent used to disperse the active ingredient in the aqueous phase.

List 3 contains those compounds which have not been fully evaluated, but which have profiles of lesser concern in the USEPA evaluation scheme. It is expected that most of these compounds will be moved to List 4 once their evaluation by the Agency is complete. There is one List 3 inert compound in the Antimycin A product: acetone a solvent which is also water miscible and aids in the dispersal of the active ingredient.

List 4 is divided into two categories. List 4A contains compounds generally regarded as safe for use in pesticide formulations and includes such compounds as corn cobs and attapulgite clay. List

4B contains those compounds that have sufficient data on file at EPA to substantiate that they can be used safely in pesticide products.

There is one compound from Inerts List 4B in the Antimycin A formulation: soybean lipids. The level of this compound is relatively low in the concentrate formulation and is further diluted when mixed with the diluent. In addition, its safety has been demonstrated by its inclusion in Inerts List 4B.

In addition to the above-mentioned review by the USEPA, all registered pesticidal end-use products (the products actually applied to the environment to control weeds or pests) must undergo a series of toxicological tests to establish their safety. Because these tests are performed on the actual end-use formulation, the effects of the "other" ingredients are effectively tested simultaneously. This toxicological screen of the "other" compounds affords an additional opportunity to examine comparative data on the active ingredient versus the end-use product to determine if there is a need to test each of them in a complete testing battery.

4.1.3 Added inert ingredients

Information on added inert ingredients is part of the Confidential Statement of Formulation, and because it is Confidential Business Information, it is not available. Some non-quantitative information is available from labels and EPA documents and will be included.

Intentionally added inert or "other" ingredients in Antimycin A formulations include: Soy lipids, diethyl phthalate, nonoxynol-9 and acetone.

4.1.4 Added synergists

There is no information or evidence that synergists are added to the antimycin A formulation; known synergists are required to be indicated on pesticide labels.

4.1.5 Nature of formulation (e.g., powder, emulsifiable concentrate)

4.2 Color

Color is an end-point observation of the product used to assist in identification.

Table 4.1 Color of Antimycin A

Formulation	Color	Citation
Antimycin A	White to yellowish	Merck, 1996
Fintrol Concentrate	Brown or Black	MSDS

4.3 Physical State

Physical state is an end-point observation of the product, solid, liquid or gaseous used to assist in identification.

Table 4.2 Physical State of Antimycin A

Formulation	Physical State	Citation
Antimycin A	Solid	Merck, 1996
Fintrol Concentrate	Liquid	MSDS

4.4 Odor

Odor is an end-point observation of the product used to assist in identification. Odor may also serve as a warning in cases where odorants are added as a safety factor.

Table 4.3 Odor of Antimycin A		
Formulation	**Odor**	**Citation**
Antimycin A	Not reported	
Fintrol Concentrate	Acetone-like	MSDS

4.5 Melting Point

The melting point is a physical end point observation used for identification of pure compounds and may provide some indication of thermal stability. Melting point is not applicable to the formulation because it is a liquid.

Table 4.4 Melting Point of Antimycin A		
Formulation	**Melting point °C**	**Citation**
Antimycin A	133-135	MP Biomedicals, 2006
Fintrol Concentrate	NA	

4.6 Boiling Point

The boiling point is a physical end point observation for identification of pure compounds. The boiling point for the active ingredient of antimycin A is undefined, because it is not a pure substance and it is a solid at room temperature.

4.7 Density, Bulk Density or Specific Gravity

Bulk density is a measure of the weight per unit volume of the product and is useful for physical identification or differentiation of two similar products. The value may also be needed to calculate application rates in some instances. Density is typically reported as grams per cubic centimeter at 25°C.

There appear to be no data on the bulk density or specific gravity of antimycin A.

4.8 Solubility

Solubility is a physical end point useful for understanding potential environmental impact. High water solubility is frequently associated with mobility and affects distribution in water and soil. This endpoint is determined for the active ingredient in a product and is typically reported as grams per 100 ml water at 25°C. The solubility of the active ingredient is minimal in water but is moderately soluble in alcohols, ether, chloroform and acetone. The formulated product is an acetone solution and is expected to miscible with water in all proportions.

Table 4.5 Solubility of Antimycin A		
Formulation	**Solubility in Water @ 25°C (g/100 ml)**	**Citation**
Antimycin A	low	Merck, 1996
Fintrol Concentrate	0.0069	USEPA, 2006a

4.9 Vapor Pressure

Vapor pressure is a physical end point useful for understanding the distribution of the active ingredient between water/soil and air. High volatility is an indication of potential impact in the air compartment. This endpoint is determined for the active ingredient in a product and is typically reported as mm mercury (Hg) at a specified temperature. In this instance, the vapor pressure was estimated using the EPA ASTER Program (ASsessment Tools for the Evaluation of Risk) (USEPA, 2006a)

Table 4.6 Vapor Pressure of Antimycin A

Formulation	Vapor Pressure @ 24.3°C (mm Hg)	Citation
Antimycin A	2.31×10^{-15}	USEPA, 2006a
Fintrol Concentrate	N/A	

4.10 Disassociation Constant

Disassociation constant is a physical end point used to assess the distribution of the pure active ingredient in aqueous media. The reported pH values indicate the environmental pH at which the active ingredient molecule will dissociate to its ionic form. In the case of Antimycin A, there are no dissociable functional groups.

4.11 Octanol/Water Partition Coefficient

Octanol/Water partition coefficient is a physical end point used to assess the potential of a compound to bioaccumulate in the environment. The value represents the ratio of product in octanol versus water at equilibrium at 25°C. Log values of K_{ow} less than 5 indicate reduced likelihood of bioaccumulation. An EPA estimation of the K_{ow} by the ECOSAR program determined the log K_{ow} for antimycin A to be 4.21.

Table 4.7 Octanol-Water Partition Coefficient of Antimycin A

Formulation	Octanol/Water Coefficient (K_{ow})	Citation
Antimycin A	4.21	USEPA, 2006DW
Fintrol Concentrate	N/A	

4.12 pH

pH is a physical end point used to identify the product and to assess the potential effect of the equilibrium in the environment. For Antimycin A and its end-use products, no data could be found on the pH.

4.13 Stability

Stability is a chemical evaluation of the product to assess the potential effect of heat, light, metals and metal ions on the active ingredient. The active ingredient is stable if stored at 0°C and protected from light (MP Biomedicals, 2006). In the case of Antimycin A formulated as Fintrol Concentrate, the product is stable for up to 36 months if stored in the unopened original glass containers. (Aquabiotics, 2004).

4.14 Oxidizing or Reducing Action

Oxidizing or reducing action is an assessment of the potential for a compound to react with common oxidizers or reducers. In the case of Antimycin A and its formulated products, there is little likelihood of such reactions occurring. The label for the formulated product indicates that for the purpose of detoxification, potassium permanganate may be used. Permanganate is considered to be a strong oxidizer and would not typically be used in standard laboratory testing. Depending on environmental conditions, excess permanganate may be required to effect detoxification of Antimycin A due to total organic carbon load in the water body (USEPA, 2006a).

4.15 Flammability

Determination of flammability is measurement of the temperature that will sustain a flame and is used to classify the product for hazard in storage and shipping. Determination of flammability is not required for technical grade products.

Table 4.8 Flash Point of Antimycin A

Formulation	Flash point °F	Citation
Antimycin A	N/A	
Fintrol Concentrate	133°F	MP Biomedicals, 2006

4.16 Explodability

Determination of explodability is measurement of the potential for a compound to explode when exposed to physical or thermal shock. Determination of explodability is not required for technical grade products. The Antimycin A molecule itself contains no explodable functional groups. The formulated product contains a high weight percentage of the flammable solvent acetone and would be expected to be explosive if the vapor concentration above the product were to reach appropriate concentrations. Care should be used when mixing and handling of the product to avoid exposure to sparks or other ignition sources.

4.17 Storage Stability

Storage stability is the physical determination of the stability of the active ingredient when stored in its commercial packaging over extended time periods, usually one to two years or more. Antimycin A products have been shown to be stable under normal storage conditions for periods of at least three years (Aquabiotics, 2004).

4.18 Viscosity

Viscosity is a physical end-point measurement used to identify the product and to assess the ability of the product to be poured or pumped. The measurement is not required on technical grade products or on solid products. The viscosity is reported in centipoise. No data were found for Fintrol Concentrate.

4.19 Miscibility

Miscibility is a physical assessment of the ability of a formulated product to mix with spray oils for use during application. Since the antimycin A aquatic product is not labeled for application in oil, this data requirement is not applicable.

4.20 Corrosion Characteristics

Corrosion characteristics require the physical observation/measurement of the effects of the product on the commercial packaging. Measurements of the weight, deformation and strength of the packaging are reported. For the Antimycin A formulations, no effect is anticipated on the glass containers for end use product packaging.

4.21 Dielectric Breakdown Voltage

Dielectric breakdown voltage is the physical measurement of the effect of an electric arc on the stability of the formulated product. This requirement applies only to formulations that are applied around electrical equipment or apparatus. As there is no likelihood of open electrical apparatus in the aquatic environment, this test is not applicable.

5. Environmental Fate

5.1 Volatilization

Volatilization data on antimycin A appear to be unavailable.

5.2 Hydrolysis

Hydrolysis refers to the chemical interaction of the pesticide with water as a mechanism of pesticide breakdown. While aqueous or aquatic (the terms are synonymous) persistence studies are sometimes conducted in natural water bodies, true hydrolysis studies are conducted in laboratories using sterile distilled or deionized water so that the chemical effects of an aqueous environment can be isolated from biological, sunlight, or sediment interactions.

Laboratory hydrolysis studies for EPA submission are typically performed with radioactive ^{14}C pure compound at three pH values (pH 5, pH 7, pH 9) corresponding to slightly acid, neutral, and mildly alkaline, respectively) in sterile water for a period of 30 days at 25°C. Sampling for breakdown products and the remaining concentration of parent material occurs at frequent intervals.

5.2.1 Half-life

Because laboratory hydrolysis studies are normally only conducted to fulfill EPA registration requirements, only one such formal study was found. In this study (Heim, 2003a, MRID 46023101 in USEPA, 2006a), hydrolysis testing was conducted 3 nominal pH values and 25°C. Antimycin A was relatively rapidly degraded with calculated half-lives of 3 days at pH 7, 15 days at pH 5 and 3 hours at pH 9.

It was also noted that there are several open literature reports of Antimycin A hydrolysis which were not conducted under the FIFRA Good Laboratory Practices, but which provide additional evidence and credibility to the limited estimated half life of the product in the aquatic environment. Half-lives of 5.5 hours at pH 7 and 20 minutes at pH 9.5 were reported by Lee (1971 in USEPA 2006a). In a separate study Hussain (1969 in USEPA 2006a) found half-lives of 46 hours at pH 7.55 and 2 minutes at pH 9. At the request of the Office of Pesticides Programs, EPA's Office of Research and Development undertook a project to develop an analytical method for antimycin and to estimate half-lives in unmixed systems and mixed systems (Kenneke, 2006 in USEPA 2006a). Kenneke found that the half-life did not vary much when tested at pH values of 4-8, but was markedly shorter at pH 9. This is comparable to findings of the other researchers

where pH 8 was not much different than pH 7, but that at pH 8.5 hydrolytic degradation was much shorter. A summary of the experimental half-lives of Antimycin A may be found in Table 5.1 below. As natural lakes would likely have a pH of approximately 7 or slightly higher in the State of Washington (WDOE, 2007) the half-life of Antimycin A as a function of hydrolysis would be less than 3 days in lakes. However, the use of antimycin in lakes is expected to be very limited because the piscicidal formulation is effective to a depth of less than 5 feet in standing waters. WDFW expects to only apply antimycin in treatments of streams and shallow ponds.

5.2.2 Degradation products

No degradation products were identified in the Heim study above. In a separate literature study, Walker suggested that Antimycin A degrades by base hydrolysis and that the major degradates were blastmycic acid, Antimycin lactone and antimycic acid (Walker, 1964). No quantitative data were provided nor were the half-lives of these products discussed. In a separate study Hussain made similar claims as to the degradation products (Hussain, 1969 in USEPA 2006a).

Table 5.1 Antimycin A Persistence in Aquatic Systems

System	Initial application rate	Half-life (DT_{50})	pH	Reference
Lab Hydrolysis	NR	15 days	5	Heim, 2003a in USEPA 2006a
		3 days	7	
		3 hours	9	
Lab Hydrolysis	NR	>7 hours	4.5 - 5.5	Lee, 1971 in USEPA 2006a
		5.5 hours	7 - 8	
		40 minutes	8.5	
		20 minutes	9.5	
Lab Hydrolysis	NR	46 hours	7.55	Hussain, 1969 in USEPA 2006a
		2 minutes	9	
Lab Hydrolysis	5 ppm	9 hours	3	Kenneke, 2006 in USEPA 2006a
		8.3 hours	4	
		10.5 hours	5	
		11 hours	6	
		7.1 hours	7	
		10 hours	8	
		3.4 hours	9	

NR = Not Reported

5.3 Aqueous photolysis

As with hydrolysis, photolysis testing is carried out in a laboratory. Vessels containing solutions of the test substance in sterile distilled or deionized water are irradiated with either a mercury vapor lamp or with natural sunlight. Identical vessels are kept in the dark for the duration of the study and also sampled in order to compensate for the effects of any hydrolysis occurring. Testing is usually carried out at 25°C, at pH 5, 7 and 9, but this is not always the case, particularly with very early studies. Other photolysis testing, such as photolysis of a pesticide on the surface of a soil, is also required by the EPA for products that might be incidentally applied to soil, as is the case for Antimycin A.

The purpose of photolysis experiments is to isolate the effect of sunlight, specifically the ultraviolet and near-ultraviolet part of the spectrum, on the degradation of a pesticide without

biological or chemical interactions. Natural sunlight's visible spectrum covers wavelengths from about 800 nm (deep red) to about 300 nm (deep violet). Generally speaking, only light in the violet and ultraviolet end of the spectrum has enough energy to initiate or influence chemical reactions ("photochemical reactions"). Air, as well as ozone, strongly filters near-ultraviolet and ultraviolet radiation, and cuts off nearly all radiation below 290 nm wavelength. Water is transparent to radiation down to approximately 180 nm (far ultraviolet), assuming that there are no suspended solids or dissolved colored material such as humic acids to impair passage of the light.

As with hydrolysis, laboratory photolysis testing is generally conducted only in response to specific EPA registration requirements. This requirement was waived for Antimycin A (EPA, 2006f)

5.4 Soil photolysis

Soil photolysis is carried out in the laboratory by exposing a thin layer of soil containing the active ingredient to either artificial or natural sunlight. The exposed soil is usually extracted to determine the amount of parent compound and any degradates that are extractable. Additional effort is typically made to do an exhaustive extraction to remove as much of the residue as practicable, especially in the case of compounds such as Antimycin A which bind strongly to soil. The soil extracts are examined to determine qualitatively and quantitatively the nature and amount of remaining parent and degradates. This requirement was waived for Antimycin A (EPA, 2006f).

5.5 Degradation and Persistence – soil

To aid the understanding of the degradation of pesticidal products in the environment, studies of aerobic and anaerobic soil metabolism are normally required for each registered product. These studies are conducted in the laboratory using radiolabeled pure active ingredient. The half-life of the parent compound is monitored as well as the formation and decline of any metabolites/degradates.

The aerobic study is typically conducted on four soil types in an aerobic (oxygen rich) environment over a sufficient time period to allow the collection of sufficient data to measure the half-life and determine the metabolic fate of the compound. The anaerobic soil metabolism study is initiated in the same manner as the aerobic study, but is made anaerobic after 30 days either by flooding with water or by a continuous purge of nitrogen to exclude the presence of oxygen in the system. Half-life of the parent compound and its metabolic fate are determined as in the aerobic study.

There are no terrestrial uses of Antimycin A currently registered and the use pattern of the product is such that direct application to terrestrial soils is highly unlikely. These requirements have been waived by USEPA (EPA, 2006f).

5.5.1 Half-life

No half lives have been calculated as there are no data from which to draw conclusions.

5.5.2 Degradation Products

No degradation products have been identified as there are no data on soil degradation from which to draw conclusions.

5.6 Degradation and persistence - aquatic systems

The disappearance of Antimycin A from a lake or other natural water body is influenced by a number of factors as discussed in section 3.1.4.3. Various water chemistry conditions, physical conditions such as temperature, adsorption to the sediment, and the extent of water currents and dilution can all have very pronounced effects on the persistence of Antimycin A.

5.6.1 Half-life and Disappearance Time

Table 5.1 above summarizes the available aqueous hydrolysis half-life data for Antimycin A and gives an estimate of the time required to achieve lowered concentration of Antimycin A in water bodies.

In addition to the hydrolysis data, a laboratory aerobic aquatic study was reported (Heim, 2003b, MRID 45895901 in USEPA, 2006a). This type of study, performed in flasks containing sediment and water obtained from ponds, lakes or streams and maintained in an aerobic state by constant aeration, is designed to generate half-life data and to examine any degradates that are formed. Calculated half-life for the water/sediment system was 23-47 days. The pH of the water in the study was 6.5.

These data are somewhat in conflict with the hydrolysis data reported above because the calculated half-life is significantly longer. One likely explanation of this difference is sorption of the molecule to the sediment particles. Using the data generated from the ASTER program (USEPA, 2006a) the K_d values for the soil would be in the range of 1-88 ml/g indicating significant adsorption to soil. These values translate to K_{oc} values in the range of 84-10,000 ml/g which is a significant level of adsorption. These data assume that equilibrium existed between the water and soil in the study, but this is a reasonable assumption due to the length of the study and the intimate contact between the water and sediment. It should be noted that this is somewhat speculative as there are no studies available to confirm the sorption characteristics of Antimycin A. Another possible explanation is that the hydrolysis data look only at antimycin A in the water, while the aerobic study includes half-life in both water and sediment.

5.6.2 Degradation Products

The Heim study above apparently concentrated only on the calculation of the half-life of Antimycin A and did not include isolation and identification of metabolites or degradation products. One can reasonably assume that at least a portion of the degradation that occurred in this study was due to hydrolysis, therefore it is likely that similar degradation products would be found. These could include: blastmycic acid, Antimycin lactone and antimycic acid (Walker, 1964; and Hussain, 1969 in USEPA, 2006a).

5.7 Microbial Degradation

There were no studies available to address microbial degradation of antimycin A.

5.8 Mobility

When a chemical is applied to soil, a potential exists for the chemical to be carried down into the soil with water movement from rain and irrigation. Pesticides exhibit a wide range of leaching potential, from those that adsorb strongly to soil particles and are not released before they break down, to those that do not adsorb significantly (or adsorb, then desorb) and will travel considerable distances down through the soil, sometimes as far as the ground water table.

Different chemicals are affected in different ways by various soil parameters such as organic matter, clay content and type, and pH.

5.8.1 Soil

No data are available. The soil adsorption/desorption study typically performed to address this data requirement has been waived by the USEPA (EPA, 2006f).

5.8.2 Sediment

Based on the data from Heim (Heim, 2003b in USEPA, 2006a) (Section 3.1.4.1 above) and the USEPA ASTER program, it is likely that sorption of Antimycin A to the sediment particles is relatively strong and will prevent the movement of the molecule in the aquatic environment. When coupled with the reported rapid hydrolysis, it is not likely that Antimycin will be available for transport through the sediment/soil column.

5.8.3 Groundwater

Groundwater data requirements have been waived by EPA. Some information can be inferred from the physical-chemical properties of antimycin A.

From the above data, it is likely that Antimycin A does not pose a significant threat to groundwater. Based on the limited available data and relying on USEPA predictive programs such as ASTER, Antimycin A is not significantly mobile in soil and sediments and is relatively strongly adsorbed to the high organic content sediments to be expected in lakes. Because Antimycin A is so readily degraded via hydrolysis, with half-lives typically ranging from a few hours to less than a few days, it is likely gone from lake water before it can be sorbed to sediments and transported into surrounding soil. Overspray onto lake shores, or exposure of treated shallow lake sediments is expected to be negligible. Even if those situations occur, Antimycin A is not significantly mobile in less-than-saturated soil situations to move beyond the immediate subsurface layers.

6. Environmental Effects

6.1 Objectives

The objective of this section is to present an overview of what ecological toxicity data are available and to list the relevant data. Subject areas to be emphasized are those related to the piscicidal uses of antimycin A, i.e., fish and other aquatic species. All higher taxa (e.g., birds, insects) will be addressed, but it is not the intent to be comprehensive for species only marginally related to piscicidal uses. This section presents data primarily from laboratory tests, along with a limited amount of outdoor, simulated pond data. Additional information on results observed under actual use conditions is in section 8 below.

6.2 Sources of Information

One primary source of information is the U.S. EPA, which has developed a number of documents related to the re-registration of antimycin A. Much of the limited amount of antimycin A toxicity data are from studies submitted to EPA and not available in the open literature. The Environmental Fate and Effects Division's final chapter (EPA, 2006a) has included fish toxicity data from Mayer and Ellersieck (1986), along with a few studies submitted directly to EPA. One compilation of data, apparently fish toxicity data related to efficacy, was submitted to EPA under

Accession No. l00135924. This compilation was said to be from 1964 and was indicated to be unpublished data and was not used by EPA because of insufficient detail (USEPA, 2006a). The contents are not known, but it noted that antimycin A was not patented as a piscicide until 1964. It is possible that these data were included in some of the extensive toxicity data generated by the U. S. Fish and Wildlife Service (FWS).

Numerous fish toxicity data were developed at the FWS Fish Control Laboratories in LaCrosse, Wisconsin (Berger et al., 1969) and are presented below. This section also includes other fish LC_{50} data from the ECOTOX data base (on-line at http://cfpub.epa.gov/ecotox/, accessed April and May 2007). EPA also accessed these data, but did not include them in the EFED chapter for the RED because none of the results showed species that are more sensitive than the coho salmon used by EFED to represent fish toxicity for antimycin A. Most of the 96-hour LC_{50} antimycin A data in this database were extracted if they were for fish native to or introduced widely into the United States; some multiple tests on the same species were combined; others were segregated to assess if the size or age of fish was a relevant factor in toxicity. EFED's one-liner database was also accessed at http://www.ipmcenters.org/Ecotox/index.cfm, but it was determined that these data were included in the EFED chapter.

6.3 Toxicity information

6.3.1 Microbes

No acute or chronic data are available to address toxicity of antimycin A to microbes. Because antimycin A is related to compounds that have been used as antibiotics, it could be expected that effects on certain bacteria are likely.

6.3.2 Algae

One ECOTOX report from 1965 indicated an endpoint of 3000 µg/L as an effect level on respiration in the green alga, *Prototheca zopfi*. (Webster & Hackett, 1965, #19933 in the ECOTOX database) Walker et al. (1964) introduced *Spirogyra* spp and phytoplankton into outdoor, vinyl "ponds" to observe for effects ancillary to testing fish. There was no gross evidence of any effects at 10 µg/L the first year or 20 µg/L the second year. Other anecdotal information from field trials indicated no effect on phytoplankton (Schnick, 1974). There are no modern, standardized data available to assess the effects of antimycin A to algae.

6.3.3 Aquatic macrophytes

No standardized data are available to address the toxicity of antimycin A to aquatic macrophytes. Early field trials and field use of antimycin A indicated no effects on macrophytes, and there appeared to be little reason for further testing. Berger et al. (1969) conducted some simulated field tests in outdoor 0.01 acre concrete pools with up to 43,000 liters of water. They observed that unidentified aquatic plants were "unharmed" by treatments with antimycin A up to 20 µg/L. Schnick (1974) reported that a variety of aquatic plants (pondweeds, knotweeds, bladderworts, water milfoil, water lilies, arrowheads, and others) were not affected by Antimycin A field studies.

Walker et al. (1964) introduced various macrophytes into outdoor, vinyl, wading pools to observe for effects ancillary to testing fish. Soils and benthic organisms were also introduced. There was no gross evidence of any effects at 10 µg/L the first year, nor at 20 µg/L the second year, Plants included *Sagittaria latifolia, Elodea canadensis, Myriophyllum heterophyllum, Potamageton nodosus, and P. pectinatus*.

6.3.4 Fish

6.3.4.1 Acute toxicity

A total of 15 acute toxicity studies of technical grade (>95% active ingredient) antimycin A on freshwater fish are contained in the EFED ecotoxicity database (Table 6.1). To ensure the risk assessment is as protective as possible of non-target fish, EPA uses the lowest scientifically defensible toxicity value available to evaluate acute risks to freshwater fish. Paddlefish (*Polyodon spathula*) were the most sensitive (LC_{50}=0.001 µg/L) freshwater fish species tested. However, the raw data used to support this endpoint could not be evaluated and therefore this value cannot be used to quantitatively assess risk The most sensitive species for which raw data could be reviewed was coho salmon (*Oncorhynchus kisutch*); therefore, the freshwater fish acute toxicity endpoint (96-hr LC_{50}) used by EPA is 0.009 µg/L. Based on the sensitivity of all freshwater fish tested with technical grade antimycin A, the compound is categorized by EPA as very highly toxic (LC_{50}<100 µg/L) to fish on an acute exposure basis.

Table 6.1. Toxicity of technical grade antimycin A to fish from USEPA (2006a)

Species	% ai	96-hour LC_{50} (µg/L)[1] (95%C.I.)	MRID/ Accession No.
Bluegill (*Lepomis macrochirus)*	95.5	.034 (0.008-0.141)	400980-01
Green Sunfish (*Lepomis cyanella)*	95.5	0.22 (0.128-0.416)	400980-01
Rainbow Trout (*Oncorhynchus mykiss)*	95.5	0.012 (0.0066-0.023)	400980-01
Cutthroat Trout (*Oncorhynchus clarki)*	95.5	0.057 (0.019-0.166)	400980-01
Coho Salmon (*Oncorhynchus kisutch)*	95.5	0.009 (0.006-0.014)	400980-01
Lake Trout (*Salvelinus namaycush)*	95.5	0.053 (0.045-0.063)	400980-01
Goldfish (*Carassius auratus)*	95.5	0.18 (0.099-0.348)	400980-01
Fathead Minnow (*Pimephales promelas)*	95.5	0.025 (0.008-0.074)	400980-01
Black Bullhead (*Ictalurus melas)*	95.5	4.8 (3.4-6.8)	400980-01
Channel Catfish (*Ictalurus punctatus)*	95.5	1.36 (1.02-0.82)	400980-01
Mosquitofish (*Gambusia affinis)*	95.5	0.19 (0.114-0.324)	400980-02
Largemouth Bass (*Micropterus salmoides)*	95.5	0.24 (0.16-0.34)	400980-03
Yellow Perch (Perca flavescens)	95.5	0.04 (0.031-0.052)	400980-04
White Crappie (*Pomoxis annularis)*	95.5	0.34 (0.27-0.42)	400980-05
Paddlefish (*Polyodon spathula)*	95.5	0.001 (0.0004-0.003)	400980-06

[1] Toxicity values are for the test material; they have not been corrected for the percent a.i.

Table 6.2 presents toxicity data for formulations of antimycin A which are unspecified but probably the formerly registered sand-coated formulations. Toxicity testing of these products (1 to 10% active ingredient) indicates that formulated products are less toxic to bluegill sunfish than the technical grade active ingredient, even when adjusted for the percent active ingredient in the formulation. This is also the case for the 10% formulation with rainbow trout, but the 1% formulation was more toxic to rainbow trout than the technical grade when corrected for the percent active ingredient. The 96-hr LC_{50} for bluegill sunfish is 1.18 µg/L for the formulated product, or 0.118 µg a.i./L whereas it is 0.034 µg/L (0.33 µg a.i./L) for the technical grade. Similarly, rainbow trout had LC_{50} values of 0.63 and 185 µg/L (0.0063 and 18.5 µg a.i./L) for formulated product while technical grade antimycin had an LC_{50} of 0.011 µg/L (10.5 µg a.i./L). The only currently registered antimycin A formulation has 23% antimycin A on a weight/weight basis.

Table 6.2. Toxicity of antimycin A formulated products to fish

Species	% ai	96-hour LC_{50} (µg/L)[1] (95%C.I.)	Toxicity Category	MRID/ Accession No.
Bluegill (*Lepomis macrochirus)*	10	1.18 (0.09-1.51)	very highly toxic	TN 901
Rainbow Trout (*Oncorhynchus mykiss)*	10	185 (134-255)	highly toxic	TN 944
Rainbow Trout (*Oncorhynchus mykiss)*	1	0.63 (0.58-0.68)	very highly toxic	TN 35
Bluegill (*Lepomis macrochirus)*	1	48-hr LC_{50} =29.5 (26-33)	very highly toxic	TN 1533
Bluegill (*Lepomis macrochirus)*	1	48-hr LC_{50} =22.5 (20.1-25.2)	very highly toxic	TN 153

[1] Toxicity values are for the test material; they have not been corrected for the percent a.i.

6.3.4.2 Comparative acute toxicology of antimycin A

There are two relevant aspects of comparative toxicology for antimycin A, species relationships and the effects of test conditions. Tables 6.1 and 6.3 show that ictalurid fish are the least sensitive, which accounts for how antimycin can be used to selectively remove unwanted fish from catfish aquaculture ponds. Other relatively insensitive species include the shortnose gar, bowfin, and goldfish, as indicated on labels. In addition, green sunfish, white crappie, and mosquitofish seem to have similar insensitivity, but are not mentioned as tolerant on the label. For a few species, such as largemouth bass, some tests indicated sensitivity and others insensitivity. There have been very few fish toxicity data generated since the initial compilations were developed. It seems possible, perhaps likely, that there are additional insensitive fish; but at present, all fish other those mentioned above should be considered as sensitive. If it is necessary, a bioassay could be used on a previously untested species to determine if that species is tolerant or sensitive.

The toxicity of antimycin A to fish is greater at higher temperatures and lower pH values. EFED presented data from field applications of antimycin where the same concentration of 0.8 µg/L killed all green sunfish when the water temperature was 22°C, but none at 12°C. Other

centrarchids showed similar effects. (USEPA, 2006a). In Table 6.3, multiple toxicity data on the same species of fish have been separated, to some extent, based upon the size of test fish. It is generally considered that small fish are more sensitive than larger fish, which is the basis for testing requirements to use small sizes (Urban and Cook, 1986). However, this does not seem to be consistent for antimycin A and several species, such as the channel catfish. The data, however, from different studies should not necessarily be considered comparable.

Berger et al. (1969) conducted numerous studies on antimycin A, under a variety of conditions, to assess its utility as a fish toxicant. For species comparisons, they generally conducted four tests under typical static test conditions. These data are summarized in Table 6.3. They found various trout species, walleye and yellow perch to be the most sensitive, bass, sunfish, minnows, and sticklebacks to be of moderate sensitivity, and shortnose gar, bowfin, and channel catfish to be of low sensitivity. White catfish and flathead catfish exhibited very low sensitivity.

After their initial tests comparing species, much of the additional work by Berger et al. (1969) was done under non-standard conditions and/or LC_{50} values were not calculated. Rather, much of their data was based only on 0% and 100% effects. They did study a number of variables, and while their data are not comparable to other studies, they did obtain results in assessing different parameters. Some of these data were generated with the technical antimycin A (96-98% a.i.) and other data were developed with the now-defunct antimycin A sand coated formulations. They indicate that the most important variable is pH, with sensitivity being much less at higher pHs. But they indicated that they did not know how much of that was because of actual toxicity at different pH values or because of degradation of antimycin A at high pHs. Temperature was also important with EC_{100}s being 2-5 times lower at 7^{o}C than at 22^{o}C. They found that antimycin is less effective in hard water. High turbidity reduced the efficacy of antimycin A. They observed that for the effects of temperature and turbidity, mortality was only retarded and not reduced at low temperatures and high turbidity.

Mayer and Ellersieck (1986) report on a number of fish tests with technical grade antimycin A, 95.5% a.i. A total of 34 tests (30 with 96-hour results) were run with rainbow trout of various stocks and sizes and when fed different diets. Although larger fish tended to be less sensitive, the results were not consistent. LC_{50}s for the largest fish were 0.115 µg/L for 46g fish and 0.089 µg/L for 107g fish; however, one test on younger fish showed an LC_{50} of 0.091.6 µg/L for 0.6g fish. Only one other LC_{50} was above 0.040 µg/L. Certain strains of rainbow trout, New Hampshire, Soap Lake, and Wytheville, were the most sensitive, with LC_{50}s being 0.007-0.009 µg/L. All tests were conducted at approximately the same hardness (40-44 mg/L), pH (7.1-7.4), and with one exception, temperature (10-12^{o}C). The single test at 17 ^{o}C did not indicate a notable difference. Of the 30 tests where 96-hour LC_{50}s were reported, the median LC_{50} was 0.014-0.016 µg/L.

Mayer and Ellersieck (1986) also reported multiple tests for other fish species. In channel catfish (N=9, median LC_{50}= 3.25 µg/L), size was the primary variable and there was a consistent pattern of larger fish being less sensitive. This was generally true for coho salmon (N=7 at 96 hours and 3 at 48 hours, median LC_{50}= 0.020 µg/L) but tests on this species varied temperature and hardness so that the size variable could not be assessed; higher temperatures did lead to greater sensitivity. Among largemouth bass (N=5, median LC_{50}>0.18 µg/L), the larger fish were more sensitive than small ones. Bluegill tests (N=17, median LC_{50}= 0.08 µg/L) also showed larger fish to be less sensitive, but there was a moderate amount of variation even among young fish of the same size. Fathead minnow tests (N=8, median LC_{50}= 0.0431 µg/L) involved temperature, hardness, and minor pH variation. There were too few perturbations of test conditions to reveal any consistent responses to the variables. The two highest LC_{50} values were at the highest and lowest

temperatures; at the most hardness, accompanied by the highest pHs, one LC_{50} was 38 µg/L, and the other was 0.165 µg/L, nearly the lowest and highest among all tests; these latter two tests were the only flow through tests reported by Mayer and Ellersieck (1986) on any species. Cutthroat trout tests (N=7, median LC_{50}= 0.083.1 µg/L) were all done with essentially the same size fish and the same water characteristics, and demonstrated approximately two-fold difference between the highest and lowest LC_{50} values

Walker et al. (1964) conducted tests with technical antimycin A to determine LC_0 and LC_{100} values for various fish species. These are not numerically comparable to LC_{50} data, but they did find that the most sensitive species they tested were gizzard shad, rainbow and brown trout, white suckers, Iowa darter, walleye, and yellow perch. Of intermediate sensitivity were northern pike, stoneroller, carp, golden shiner, fathead minnow, bigmouth buffalo, brook stickleback, green sunfish, pumpkinseed, bluegill, longear sunfish, largemouth bass, and white crappie. Goldfish, black and yellow bullheads, and channel catfish were the most resistant.

EFED (USEPA, 2006a) noted that efficacy in field applications of antimycin A appears to be related to temperatures and pH values, as was also observed in laboratory tests. In addition, a high stream gradient reduces efficacy, but this results most likely from reduced antimycin A concentrations in such waters, rather than from any toxicological considerations.

Table 6.3 Acute toxicity of antimycin A to fish from EPA's ECOTOX database

Species	Age/ Size	Test Type	Test material/ % Active ingredient	Toxicity value (LC_{50})	Reference[1]
American Eel *(Anguilla rostrata)*	97 mm	96 hr	100%	0.28 µg/L	592 Hinton
American Eel (*Anguilla rostrata*)	55 mm	96 hr	100 %	0.09 µg/L	593 Hinton
American Eel (*Anguilla rostrata*)	114-340 g	96 hr	NR	3.0 µg/L	456 Hinton
Black bullhead (*Ictalurus melas*) 4 tests	2.2-2.4g	S 96 hr	NR	21-88 µg/L	Berger et al, (1969)
Black bullhead (*Ictalurus melas*) 2 tests	1.2 g	S 96 hr	95.5%	4.8-7.5 µg/L	Mayer and Ellersieck (1986)
Bluegill (*Lepomis macrochirus*) 4 tests	0.8-2.5g	S 96 hr	96-98%	0.06-0.5 µg/L	Berger et al, (1969)
Bluegill (*Lepomis macrochirus*)	NR	96 hr	Tech	0.144 µg/L	904 Marking
Bluegill (*Lepomis macrochirus*)	NR	96 hr	Tech	0.157 µg/L	958 Howland
Bluegill (*Lepomis macrochirus*) 14 tests	0.6-4.8g	S 96 hr	95.5%	0.0339-0.159 µg/L	Mayer and Ellersieck (1986)
Bluegill (*Lepomis macrochirus*)	9.7g	S 96 hr	95.5%	0.18 µg/L	Mayer and Ellersieck (1986)
Bluegill (*Lepomis macrochirus*)	20g	S 96 hr	95.5%	0.197 µg/L	Mayer and Ellersieck (1986)
Bowfin (*Amia calva*)	3wk fry	S 96 hr	96-98%	0.13 µg/L	Berger et al, (1969)

Table 6.3 Acute toxicity of antimycin A to fish from EPA's ECOTOX database					
Species	**Age/ Size**	**Test Type**	**Test material/ % Active ingredient**	**Toxicity value (LC_{50})**	**Reference[1]**
Bowfin (*Amia calva*)	6wk fry	S 96 hr	96-98%	0.24 µg/L	Berger et al, (1969)
Bowfin (*Amia calva*)	8wk fry	S 96 hr	96-98%	0.35 µg/L	Berger et al, (1969)
Brook stickleback (*Culaea inconstans*) 4 tests	1.1g	S 96 hr	96-98%	0.04-0.55 µg/L	Berger et al, (1969)
Brook trout (*Salvelinus fontinalis*) 2 tests	1.5g	S 96 hr	96-98%	0.03-0.06	Berger et al, (1969)
Carp (*Cyprinus carpio*) 4 tests	2.0 g	S 96 hr	96-98%	0.12-0.43	Berger et al, (1969)
Carp (*Cyprinus carpio*) 7 tests	2.2 g	S 96 hr	NR	0.12-0.43	Berger et al, (1969)
Channel catfish (*Ictalurus punctatus*) 2 tests	0.1-0.15g	S 96 hr	95.5%	1.36-1.58 µg/L	Mayer and Ellersieck (1986)
Channel catfish (*Ictalurus punctatus*) 4 tests	1.4-2.3g	S 96 hr	95.5%	3.3-4.36 µg/L	Mayer and Ellersieck (1986)
Channel catfish (*Ictalurus punctatus*)	5.0 g	S 96 hr	95.5%	4.61 µg/L	Mayer and Ellersieck (1986)
Channel catfish (*Ictalurus punctatus*) 2 tests	9.7 g	S 96 hr	95.5%	>3,2 µg/L	Mayer and Ellersieck (1986)
Channel catfish (*Ictalurus punctatus*)	18.0 g	S 96 hr	95.5%	6.86 µg/L	Mayer and Ellersieck (1986)
Channel catfish (*Ictalurus punctatus*)	NR	96 hr	NR	14.7 µg/L	904 Marking
Channel catfish (*Ictalurus punctatus*)	Fry (few hours)	S 96 hr	NR	1.0 µg/L	Berger et al, (1969)
Channel catfish (*Ictalurus punctatus*) 4 tests	1.9g	S 96 hr	96-98%	5.2-10.5 µg/L	Berger et al, (1969)
Coho salmon (*Oncorhynchus kisutch*) 6 tests	0.5-11.1g	S 96 hr	95.5%	0.009-0.021 µg/L	Mayer and Ellersieck (1986)
Coho salmon (*Oncorhynchus kisutch*)	19.4 g	S 96 hr	95.5%	0.06 µg/L	Mayer and Ellersieck (1986)
Cutthroat trout (*Oncorhynchus clarki*) 7 tests	1-1.1g	S 96 hr	95.5%	0.057-0.112 µg/L	Mayer and Ellersieck (1986)
Cutthroat trout (*Oncorhynchus clarki*)	NR	96 hr	NR	0.11 µg/L	2879 Swedburg
Fathead minnow (*Pimephales promelas*) 2 tests	NR	S 96 hr	23%	0.018-0.27 µg/L	15277 Gilderhus
Fathead minnow (*Pimephales promelas*) 2 tests	0.5 g	F 96 hr	95.5%	0.038-0.165 µg/L	Mayer and Ellersieck (1986)
Fathead minnow (*Pimephales promelas*) 6 tests	0.5-2.1g	S 96 hr	95.5%	0.038-0.265 µg/L	Mayer and Ellersieck (1986)

Table 6.3 Acute toxicity of antimycin A to fish from EPA's ECOTOX database					
Species	**Age/ Size**	**Test Type**	**Test material/ % Active ingredient**	**Toxicity value (LC_{50})**	**Reference[1]**
Fathead minnow (*Pimephales promelas*) 4 tests	0.7-1.7g	S 96 hr	96-98%	0.06-0.21 µg/L	Berger et al, (1969)
Flathead catfish (*Pylodictus olivarus*)	"Adult"	S 96 hr	NR	182 µg/L	Berger et al, (1969)
Flathead catfish (*Pylodictus olivarus*)	Fingerling 5.8 in	S 96 hr	NR	54 µg/L	Berger et al, (1969)
Freshwater Drum (*Aplodinotus grunniens*) 3 tests	3.3g	S 96 hr	96-98%	0.02-0.14 µg/L	Berger et al, (1969)
Goldfish (*Carrasius auratus*) 4 tests	0.4-2.3g	S 96 hr	96-98%	0.2- 1.0 µg/L	Berger et al, (1969)
Goldfish (*Carrasius auratus*) 3 tests	0.9-1.0g	S 96 hr	95.5%	0.18-0.2 µg/L	Mayer and Ellersieck (1986)
Green sunfish (*Lepomis cyanella*) 4 tests	0.6-0.7g	S 96 hr	96-98%	0.11-0.50 µg/L	Berger et al, (1969)
Green sunfish (*Lepomis cyanella*)	1.1 g	S 96 hr	95.5%	0.22 µg/L	Mayer and Ellersieck (1986)
Lake trout (*Salvelinus namaycush*)	4 g	S 96 hr	96-98%	0.07 µg/L	Berger et al, (1969)
Largemouth bass (*Micropterus salmoides*) 3 tests	0.8 g	S 96 hr	96-98%	0.09-0.14 µg/L	Berger et al, (1969)
Largemouth bass (*Micropterus salmoides*)	0.6 g	S 96 hr	95.5%	0.237 µg/L	Mayer and Ellersieck (1986)
Largemouth bass (*Micropterus salmoides*) 4 tests	6.1-20.5 g	S 96 hr	95.5%	0.063-0.252 µg/L	Mayer and Ellersieck (1986)
Longear sunfish (*Lepomis megalotis*)	1.0g	S 96 hr	96-98%	0.08 µg/L	Berger et al, (1969)
Northern Pike (*Esox lucius*) 4 tests	0.8-2g	S 96 hr	96-98%	0.11-0.55 µg/L	Berger et al, (1969)
Northern redbelly dace (*Phoxinus eos*) 4 tests	1.1-2.4g	S 96 hr	96-98%	0.09-0.52 µg/L	Berger et al, (1969)
Paddlefish (*Polyodon spathula*)	0.01g	S 96 hr	95.5%	0.001 µg/L	Mayer and Ellersieck (1986)
Pumpkinseed (*Lepomis gibbosus*) 4 tests	1.3-1.4 g	S 96 hr	96-98%	0.05-0.24 µg/L	Berger et al, (1969)
Rainbow trout (*Oncorhynchus mykiss*) 3 tests	1.5-1.6 g	S 96 hr	96-98%	0.3-0.8 µg/L	Berger et al, (1969)
Rainbow trout (*Oncorhynchus mykiss*)	12 day fry	S 96 hr	NR	0.03 µg/L	Berger et al, (1969)
Rainbow trout (*Oncorhynchus mykiss*) 3 tests	"fingerling"	S 96 hr	NR	0.03-0.1 µg/L	Berger et al, (1969)

Table 6.3 Acute toxicity of antimycin A to fish from EPA's ECOTOX database					
Species	**Age/ Size**	**Test Type**	**Test material/ % Active ingredient**	**Toxicity value (LC_{50})**	**Reference[1]**
Rainbow trout (*Oncorhynchus mykiss*)	5-day fry	S 96 hr	NR	0.04 µg/L	Berger et al, (1969)
Rainbow trout (*Oncorhynchus mykiss*)	60-d fry	S 96 hr	NR	0.04 µg/L	Berger et al, (1969)
Rainbow trout (*Oncorhynchus mykiss*)	44-d fry	S 96 hr	NR	0.04 µg/L	Berger et al, (1969)
Rainbow trout (*Oncorhynchus mykiss*)	18-d fry	S 96 hr	NR	0.05 µg/L	Berger et al, (1969)
Rainbow trout (*Oncorhynchus mykiss*)	NR	96 hr	Tech	0.032 µg/L	958 Howland
Rainbow trout (*Oncorhynchus mykiss*)	NR	96 hr	Tech	0.048 µg/L	904 Marking
Rainbow trout (*Oncorhynchus mykiss*) 30 tests	0.7-107g	S 96 hr	95.5%	0.007-0.115 µg/L	Mayer and Ellersieck (1986)
Rainbow trout (*Oncorhynchus mykiss*)	0.5 g	S 96 hr	NR	0.032 µg/L	13025 Thompson
Redear sunfish (*Lepomis microlophus*)	1.3 g	S 96 hr	96-98%	0.09 µg/L	Berger et al, (1969)
Shortnose gar (*Lepisosteus platostomus*)	0.8 g	S 96 hr	96-98%	0.48 µg/L	Berger et al, (1969)
Smallmouth bass (*Micropterus dolomieu*) 3 tests	0.5 g	S 96 hr	96-98%	0.04-0.06 µg/L	Berger et al, (1969)
Walking catfish (*Clarias batrachus*)	3.8g	S 96 hr	95.5%	15 µg/L	Mayer and Ellersieck (1986)
Walleye *(Stizostedion vitreum vitreum*) 2 tests	0.7 g	S 96 hr	96-98%	0.02-0.04 µg/L	Berger et al, (1969)
Western mosquitofish (*Gambusia affinis*)	0.6 g (resistant)	S 96 hr	95.5%	0.564 µg/L	Mayer and Ellersieck (1986)
Western mosquitofish (*Gambusia affinis*)	0.6 g (non-resistant)	S 96 hr	95.5%	0.192 µg/L	Mayer and Ellersieck (1986)
White crappie *(Pomoxis annularis*)	1.5 g	S 96 hr	95.5%	0.34 µg/L	Mayer and Ellersieck (1986)
Yellow perch (*Perca flavescens*) 4 tests	0.5-2.0 g	S 96 hr	96-98%	0.03-0.12 µg/L	Berger et al, (1969)
Yellow perch (*Perca flavescens*)	0.7 g	S 96 hr	95.5%	0.04 µg/L	Mayer and Ellersieck (1986)

[1] All references without a date are from the ECOTOX database. Only the first author is mentioned, but the ECOTOX number is unique to each publication. Details for these are included at the end of the reference section under ECOTOX.

For estuarine/marine fish, USEPA reported only one acute toxicity study on the spot (*Leiostomus xanthurus*) with technical grade antimycin A. The 48-hr LC_{50} was determined to be 0.23 µg/L.

6.3.4.3 Chronic toxicity

No chronic toxicity data on antimycin A are available for freshwater or estuarine/marine fish either from EPA material or in the open literature.

6.3.5 Aquatic invertebrates

Although there are numerous toxicity data for antimycin A on fish to test efficacy and comparative sensitivity, the typical laboratory toxicity data for antimycin A and aquatic invertebrates are quite limited. Most of the information on aquatic invertebrates was gathered in conjunction with actual use of antimycin A in fish control projects, and most of these data are more anecdotal than quantitative.

6.3.5.1 Acute toxicity

Antimycin A exhibits very high acute toxicity to aquatic invertebrates, based upon the most sensitive species. The most sensitive aquatic invertebrate, (*Gammarus fasciatus*), with an LC_{50} of 0.008 µg/L, was as sensitive as the coho salmon. Other invertebrate testing included by EFED did not establish LC_{50}/EC_{50} endpoints, with sow bugs being greater than 1 µg/L and *Daphnia magna* being less than 10 µg/L (according to Table 6.4, but probably less than 5 µg/L, based upon the text indicating that there was greater than 50% mortality at all test levels). In general, the data in the ECOTOX database did not support the extreme sensitivity determined in the EPA review, but did show high sensitivity of amphipods and midges. Crayfish, shrimp, and mollusks had LC_{50} values greater than 5 µg/L, which is frequently the maximum rate used in fish control projects.

Table 6.4 Toxicity of technical grade antimycin A to aquatic invertebrates from USEPA (2006a)

Species	% ai	96-hour LC_{50} (µg/L)[1] (95%C.I.)	Toxicity Category	MRID/ Accession No.	Study Classification
Waterflea (*Daphnia magna)*	95.5	48 hr EC_{50}<10 (5-10)	very highly toxic	400980-01	Supplemental
Scud (*Gammarus fasciatus)*	95.5	0.008 (0.0058-0.011)	very highly toxic	400980-01	Supplemental
Aquatic Isopod (*Asellus brevicaudus)*	95.5	>1.0	very highly toxic	400980-01	Supplemental

[1] Toxicity values are for the test material; they have not been corrected for the percent a.i.

In addition to data on freshwater invertebrates used by EPA, toxicity data were found in the ECOTOX data base and are presented in Table 6.5. The data for aquatic invertebrates are rather limited, compared to fish, but do suggest that most aquatic invertebrates are less sensitive than the more sensitive fish.

Table 6.5 Acute toxicity to aquatic invertebrates from the ECOTOX data base

Species	Age/ Size	Test Type	Test material/ % Active ingredient	Toxicity value (LC_{50})	Reference
Aquatic Arthropods					
Scud (*Gammarus pseudolimnaeus*)	NR	96 hr	23%	7.2 µg/L	6985 Baumann

Table 6.5 Acute toxicity to aquatic invertebrates from the ECOTOX data base					
Species	**Age/ Size**	**Test Type**	**Test material/ % Active ingredient**	**Toxicity value (LC_{50})**	**Reference**
Scud (*Gammarus pseudolimnaeus*)	NR	96 hr	23%	9.0 µg/L	6985 Baumann
Scud (*Hyalella azteca*)	NR	96 hr	23%	1.4 µg/L	6985 Baumann
Midge (*Chironomus tentans*)	4th instar	S 96 hr	95.5%	0.146 µg/L	5776 Kawatsi
Grass shrimp (*Palaemonetes kadiakensis*)	mature	S 96 hr	95.5%	3000-6000 µg/L	Mayer and Ellersieck (1986)
Crayfish (*Cambarus sp.*)	NR	4 days	NR	10 µg/L	Walker et al., 1964
Crayfish (*Procambarus sp.*)	Juvenile (19 mm)	4 days	23%	168 µg/L	5976 Brown
Crayfish (*Procambarus sp.*)	Molted juvenile (30 mm)	4 days	23%	175 µg/L	5976 Brown
Crayfish (*Procambarus sp.*)	Molted juvenile (8mm)	4 days	23%	39 µg/L	5976 Brown
Crayfish (*Procambarus sp.*)	Molted juvenile (19mm)	4 days	23%	60 µg/L	5976 Brown
Crayfish (*Procambarus sp.*)	Juvenile (30 mm)	4 days	23%	735 µg/L	5976 Brown
Crayfish (*Procambarus sp.*)	Juvenile (8 mm)	4 days	23%	68 µg/L	5976 Brown
Crayfish (*Procambarus sp.*)	eggs	4 days	23%	5-15 µg/L	5976 Brown
Mollusks					
Asiatic clam (*Corbicula manilensis*)	1.0-2.7g	F 96 hr	23%	86 µg/L	418 Chandler
Asiatic clam (*Corbicula manilensis*)	1.0-2.7g	S 96 hr	23%	65 µg/L	418 Chandler
Fat mucket (lamp-mussel) (*Lampsilis siliquoidea*)	NR	S 27 days	23%	5-15 µg/L	2471 Antonioni
Spike (mussel) (*Elliptio dilatata*)	NR	S 27 days	23%	5-15 µg/L	2471 Antonioni
Snail (*Viviparus bengalensis*)	NR	NR 96 hr	95%	5.8 µg/L	Gupta and Durve, 1983
Other					
Flatworm (*Dugesia dorotocephala*)	NR	S 8 days	23%	15 µg/L	6985 Baumann

Walker et al. (1964) conducted laboratory tests to determine EC_0 and EC_{100} concentrations of antimycin A. They found that waterfleas survived 0.5 and 1.0 µg/L at 12°C, but died in 48 hours at 10 µg/L and in 24 hours at 100 µg/L. At 22°, they still survived at 0.1µg/L, but died in 48 hours at 0.5 µg/L and after 24 hours at 10 µg/L. Crayfish were unaffected after 96 hours at 10 µg/L and 12°C. Damselfly nymphs were unaffected at 50 µg/L and either 12°C or 22°C, but died at higher concentrations. Based upon simulated field studies in outdoor ponds, they concluded that there were no significant changes in 15 invertebrate taxa, including horsehair worms, aquatic earthworms, leeches, amphipods, mayflies, damselflies, dragonflies, water bugs, caddisflies, water beetles, mosquitoes, midges, biting midges, soldierflies, and snails after treatment with 10 µg/L of antimycin A the first year or at 20 µg/L the second year.

Berger et al. (1969) conducted some simulated field tests in outdoor 0.01 acre concrete pools with up to 43,000 liters of water. They observed that damselfly naiads were "unharmed" by treatments with antimycin A up to 20 µg/L. Schnick (1974) reported that in a variety of field trials, some effects were observed in certain taxa of aquatic invertebrates when used at concentrations of 5 µg/L and below. Largely anecdotal observations indicated that when there were effects on aquatic invertebrates, they were relatively short-lived. This was considered an important consideration in rehabilitation projects because the aquatic invertebrates are necessary to proper establishment of fish populations. Amphipods appear to be the most sensitive group of invertebrates, and they and cladocerans, copepods, and ephemeropteran (mayfly) and trichopteran (caddisfly) insects were most likely to disappear temporarily after antimycin A treatments. Protozoans, rotifers, nematodes, nematomorphs, annelids, ostracod and decapod (shrimp and allies) crustaceans, snails, bivalve mollusks, and insects in the orders of plecoptera (stoneflies), odonata (dragonflies, et al.), hemiptera (true bugs), coleoptera (beetles), diptera (flies) are generally not affected by antimycin A.

Field observations have indicated that, while aquatic invertebrates will be killed by antimycin A field applications, the effects are much more limited than for fish (Pfeifer et al., 2001). When antimycin A is applied at concentrations much above 10 µg/L, effects on invertebrates have been obvious, but generally of short duration, typically recovering in several months to a year (Jacobi and Degan, 1977; Houf and Campbell, 1977; Dinger and Marks, 2006). When treatment concentrations were at 10 µg/L and below, effects on invertebrates were not observed (Cerreto, 2004) or minimal and brief (Houf and Campbell, 1977; Moore et al, 2005).

Table 6.6 shows that antimycin A is very highly toxic to estuarine/marine invertebrates from acute exposure, based on a pink shrimp 96-hr LC_{50} of 24 µg/L. Acute toxicity data were also available on the Eastern oyster, *Crassostrea virginica,* showing that antimycin is very highly toxic to mollusks as well. Although the toxicity of antimycin A to these species is categorized as "very highly toxic," the data indicate that these species are several orders of magnitude less sensitive than the freshwater *Gammarus fasciatus*.

Table 6.6. Toxicity of technical grade antimycin A to estuarine/marine invertebrates from USEPA (2006a)

Species	% ai	96-hour LC_{50} (µg/L)[1] (95%C.I.)	Toxicity Category	MRID/ Accession No.	Study Classification
Pink Shrimp (*Penaeus duorarum)*	95.5	48 hr EC_{50}=24	very highly toxic	402284-01	Supplemental
Blue Crab	95.5	48-hr	highly toxic	402284-01	Supplemental

(*Callinectes sapidus)*		LC_{50}>100			
Eastern Oyster (*Crassostrea virginica)*	95.5	62	very highly toxic	402284-01	Supplemental

[1] Toxicity values are for the test material; they have not been corrected for the percent a.i.

6.3.5.2 Chronic toxicity

No chronic toxicity data on antimycin A were located for either freshwater or estuarine/marine invertebrates.

6.3.6 Amphibians

There are limited data available on the effects of antimycin A on amphibians. Grisak et al. (2006) found that two adult frog species were not significantly affected at concentrations below 10 µg/L. Tailed frogs did exhibit 15% mortality at 7.5 µg/L after 96 hours. But the authors noted that at concentrations typically used for fish control (5-10 µg/L), it required 72 hours and 3 daily renewals of antimycin concentrations to cause even 5% mortality.

There are very limited additional data on amphibians (Table 6.7). Walker et al. (1964) determined 100% effect and 0% effect levels for antimycin A on adult tiger salamanders and tadpole bullfrogs. Concentrations causing 100% mortality were 600 µg/L for the salamander and 40 µg/L for the bullfrog tadpoles; corresponding concentrations causing no mortality were 80 µg/L for the salamander and 20 µg/L for the bullfrog. There were no intermediate test concentrations so the minimum LC_{100} could be lower and/or the maximum LC_0 could be higher. The test duration was 4 days for the salamander, but only one day for the bullfrog tadpoles. LC_{50} values were not calculated but would be between the no effect and 100% effect levels. The salamanders would not be sensitive to antimycin A treatments and the bullfrog tadpoles would not be sensitive at typical treatment levels of 5-10 µg/L.

Table 6.7 Toxicity of antimycin A to amphibians, from the ECOTOX data base

Species	Age/ Size	Test Type	Test material/ %A.I.	Toxicity value (LC_{50}) [1]	Reference
Tiger Salamander (*Ambystoma tigrinum*)	Adult	S 96 hr	Tech 90%	600 µg/L 100% mort 80 µg/L 0% mort	Walker et al., 1964
Bullfrog (*Rana catesbeiana*)	Tadpole	S 96 hr	Tech 90%	40 µg/L 100% mort 20 µg/L 0% mort	Walker et al., 1964
Columbia spotted frog (*Rana luteiventris*)	Adult (2.12g)	S 96 hr	Fintrol 23%	192 µg/L	Grisak, et al 2006
Long-toed salamander (*Ambystoma macrodactylum*)	Adult (1.61g)	S 96 hr	Fintrol 23%	7.5 µg/L 0% mort	Grisak, et al 2006
Long-toed salamander (*Ambystoma macrodactylum*)	Larvae (1.58g)	S 96 hr	Fintrol 23%	81.7 µg/L	Grisak, et al 2006
Tailed frog (*Ascaphus truei*)	Adult (1.04g)	S 96 hr	Fintrol 23%	13.7 µg/L	Grisak, et al 2006

[1] Data are LC_{50} data except where noted

6.3.7 Sediment organisms

Most of the invertebrates included in tables 6.4 and 6.5 are benthic organisms living in or on sediments. *Daphnia magna* is the only one of these species considered a water column invertebrate, although some of the decapods may not be exclusively associated with benthic habitats. It is, however, unlikely that these organisms were used in a test system that includes sediment materials.

6.3.8 Toxicity to Birds

6.3.8.1 Acute toxicity

The only avian toxicity data available for antimycin A are acute oral LD_{50} data on a formulated product with no percentage of active ingredient stated for the test formulation. Based upon the results presented in Table 6.8, antimycin A is categorized as very highly toxic to waterfowl (mallard duck LD_{50}=2.9 mg/kg bw) and highly toxic to upland game birds (bobwhite quail LD_{50}=39 mg/kg bw). No sub-acute dietary toxicity data of antimycin A have been found for birds.

Table 6.8 Acute oral toxicity of an unidentified formulation of antimycin A to birds from USEPA (2006a)

Species	% ai	LD_{50} (mg/Kg)	Toxicity Category	MRID/ Accession No.
Mallard duck (*Anas platyrhynchos*)	NR	LD_{50}: 2.9 mg/kg	very highly toxic	135924
Bobwhite quail (*Colinus virginianus*)	NR	LD_{50}: 39 mg/kg	highly toxic	135924

NR = Not reported

6.3.8.2 Chronic toxicity

No chronic toxicity data are available for birds.

6.3.9 Toxicity to Mammals (from USEPA, 2006d)

6.3.9.1 Acute toxicity

No toxicity data are available on technical grade antimycin A; however, the acute oral LD_{50} for Fintrol® Concentrate (20% solution) is 286 mg/kg for male and 361 mg/kg for female rats.

6.3.9.2 Chronic toxicity

In a 90-day rat study, the LOAEL was determined to be 0.5 mg/Kg/day based on diarrhea and soft stools. A NOAEL was not established. The increased incidence of diarrhea and soft stool probably results from effects on the intestinal flora and is consistent with the antibiotic origins of antimycin A.

6.3.10 Terrestrial plants

There are no phytotoxicity data for antimycin A because these were waived on the basis of only aquatic use. There is no apparent effect on aquatic macrophytes (section 6.3.3)

and no reason to expect any toxicity to terrestrial plants. The likelihood of exposure to terrestrial plants is minimal.

7. Ecological Exposure Assessment

7.1 Routes of exposure

Antimycin A applications are made only by ground methods. The Fintrol label recommends the use of a boat bailer or spray equipment, but then further indicates that "sprays are useful at one foot" and that boat bailer and drip tubes are useful at greater depths when applied into the propeller wash of the boat. Backpack sprayers may also be used. In general, the ground applications should not result in off-site exposure. WDFW expects that they will use antimycin A with drip stations and backpack/mobile sprayers as the primary means of application. The drip station procedures are similar to those described by Finlayson, et al., 2000 for rotenone. (J. Anderson, email communication, June 13, 2007)

7.1.1 Aquatic plants and algae

Applications are made directly to water. Thus, the primary route of exposure for aquatic plants and algae would be through antimycin A concentrations in the water. For applications made above the water, exposure to emergent plants could result from direct application. Based upon the K_d, the soil-water adsorption coefficient, of antimycin A, it would be expected that antimycin A would adsorb not only to soils and sediments, but also to algae and plants in the water. While data are lacking to support any definitive conclusions, the likelihood that antimycin A would penetrate into plant cells and tissues seems low. Gilderhus (1982) found that toxicity of antimycin A to fish was reduced by the presence of bentonite clay particles or by the presence of *Elodea canadensis*, a rooted aquatic macrophyte. The toxicity reductions seem most likely to be from reduced bioavailability resulting from adsorption.

7.1.2 Fish and other aquatic vertebrates; aquatic invertebrates

Applications are made directly to water. As with algae and aquatic plants, the primary route of exposure would be from antimycin A in the water column. Fish and aquatic arthropods would take up antimycin A fairly easily through their gills. Some dermal or oral uptake could also occur, but would be considerably less than would occur through the gills. The same routes would apply to gilled larval or neotenic stages of amphibians. Concentrations to which fish and water column invertebrates would be exposed would be determined by the objectives of the treatment project; models to predict exposure are unnecessary. There are no data available to assess exposure to benthic organisms, and any toxicity data developed without sediments in the test system may not accurately represent the potential *in situ* effects of antimycin A on these organisms. It is possible that degradation of antimycin A could occur rapidly enough to result in negligible exposure in sediments.

Other vertebrates that may occur in or on the water would be exposed to antimycin A either through dermal uptake, which is expected to be low (USEPA, 2006d), or more likely, through ingestion of treated water or food items with antimycin A residues. EPA reports that fish killed by antimycin A had tissue residues as high as 172 µg antimycin/kg.

7.1.3 Terrestrial organisms

Terrestrial organisms that would be exposed to antimycin A are those that consume treated water or prey organisms from that water**.** Some exposure could occur through contact with the water, but this would be relatively minor for terrestrial animals. With no aerial applications, the exposure of terrestrial organisms feeding on land would be negligible.

7.2 Estimated concentrations of antimycin A

Based upon label directions, the maximum estimated environmental concentration (EEC) of antimycin A would be the same as the maximum target concentration, 25 µg/L. The 25 µg/L concentration is used to achieve a complete kill, and may not be that high in many eradication projects. Most applications of antimycin A appear to be selective and lower application rates will result in concomitantly lower aquatic concentrations. During applications, there could be areas with somewhat higher amounts of antimycin until it has been dispersed throughout the water column

7.2.1 Water column – lentic

Concentrations in the water column of lakes, ponds, and reservoirs would be a maximum of 25 µg/L under the proposed labeling. Specific goals of a fish rehabilitation project would often lead to lower application rates and resulting lower concentrations. Applications with boat bailers or drip tubes may be mixed quickly from the action of boat engine propellers, especially in shallow lakes. WDFW expects that drip stations and backpack/mobile sprayers will be the primary means of application. The drip station procedures are similar to those described by Finlayson, et al., 2000 for rotenone. It is unlikely that antimycin would be used in deeper lakes: WDFW has indicated that they would use it only in shallow ponds.

7.2.2 Water column – lotic

As with lentic waters, the maximum rate for antimycin A in lotic waters is 25 µg/L. Selective treatments at lower rates are common. Because antimycin is apparently non-repellant to fish, there would be no "barrier" treatments of lotic waters above treated lakes. Drip stations are the most likely means of application. Mixing in lotic waters would likely be quick for smaller streams; it does not appear that it would be efficacious to use antimycin A in larger rivers, especially those with inaccessible side channels and backwaters.

7.2.3 Sediments

No data are available; some inferences may be made based upon physical-chemical properties. Based on Kd values, it appears likely that antimycin A would adsorb to sediments. The K_{oc} values suggest even greater adsorption to sediments with high organic carbon content.

7.2.4 Adjacent terrestrial areas

Based upon the application methods of direct application into water or backpack sprayer in shallow water, no exposure of adjacent terrestrial areas is expected. Aerial application is specifically not recommended on current labels.

7.3 Persistence and duration of residues

USEPA (2006a) reports that the fate and transport of antimycin A are poorly known. Antimycin does not appear to persist in the environment, but the mechanisms of its degradation and transport

are unclear. Investigation of these mechanisms has been hindered by the unavailability of analytical methods for detecting low amounts of antimycin A.

7.3.1 Water

The persistence of antimycin in water is uncertain, but is expected to be relatively brief. The label indicates that the compound should degrade to below levels of fish toxicity in about one week. But this is determined by a bioassay of sensitive fish rather than chemical analysis, so quantification is not available.

The hydrolysis half-life data in the laboratory are somewhat equivocal (see section 5.2), with one study showing a 15-day half life at pH 5 and a 3-day half life at pH 7 (Heim 2003a in USEPA, 2006a), but another study showing the half life to not exceed 11 hours at any pH. All studies indicate a short half life, ranging from 2 minutes to 3.4 hours at pH 9. The short half life at pH 9 has probably been the reason that antimycin is considered to degrade rapidly in alkaline waters. But this may not actually be the case for all alkaline waters. The hydrolysis study in the field by Lee et al. (1971 in USEPA 2006a) found that antimycin persisted for 5.5 hours at pH 7-8, but persisted only 40 minutes at pH 8.5. Similarly, the study by Kenneke (2006 in USEPA, 2006a) found a half life of 10 hours at pH 8, but only 3.4 hours at pH 9. Hussain (1969 in USEPA 2006a) found a half life of 46 hours at pH 7.55, but only 2 minutes at pH 9. These data all suggested rapid hydrolytic degradation at strongly alkaline pHs of 8.5 and above, but that persistence at slightly alkaline pHs at 8 and below may not be much shorter than in neutral or acidic waters.

USEPA (2006a) did evaluate a study on aerobic aquatic metabolism that had a half-life in the range of 23 to 47 days in pH 6.5 water. They speculated that this longer half life could be due to adsorption to sediments that might shield the antimycin A from hydrolytic degradation. It is not known how these laboratory results would apply in field applications to water bodies where sediments would be present.

When used in streams with steep gradients, antimycin is rapidly degraded, apparently by the physical action in the tumbling water (Moore et al., 2005) or possibly by oxygenation (Pfeiffer et al., 2003).

7.3.2 Sediment

Because of the lack of analytical methods to assess residues in the field, the persistence of antimycin A residues in the sediments is unknown. Adsorption to sediments is expected and could be strong, but the rate of degradation or other loss of antimycin A sorbed to sediments cannot be determined. As with many other fate and transport parameters for antimycin A, non-quantitative information obtained from bioassays would provide an indication of when sediment residues fell below toxic levels.

7.3.3 Soil

No data are available on persistence of antimycin A in soils. Terrestrial fate and transport data have been waived by EPA because the sole use of antimycin is for application directly to water.

7.4 Bioconcentration and Bioaccumulation

No data are available. Using standard methods developed for assessing the potential of chemical compounds for persistence, bioaccumulation, and toxicity, i.e., the PBT profiler, USEPA (2006a)

estimated the bioconcentration factor (BCF) for antimycin A to be 350x. At this predicted level of bioconcentration, EPA does not have a concern for either bioconcentration or bioaccumulation, the latter of which includes uptake through ingestion as well as the gill uptake for bioconcentration.

7.4.1 Within organisms

Maximum residues of antimycin A found in the tissues of rainbow trout killed by the compound were 172 µg antimycin/Kg body weight, or 0.172 ppm (USEPA, 2006a). No data were found relating to the duration of residues within exposed organisms, nor were any data found on residues of antimycin A in living fish.

7.4.2 Accumulation and other food chain transfer

No data are available. Based upon the estimated BCF of 350x, EPA does not have a concern for bioaccumulation of antimycin A (USEPA, 2006a).

7.5 Ground and well water considerations

7.5.1 General aspects of groundwater and wells.

There are very few data that directly apply to the potential for ground water exposure with antimycin A. The situation is also complicated by the lack of good methods for analyzing residues of antimycin A. Based on its physical-chemical properties, antimycin A does not appear to be a ground water concern. Its tendency to adsorb to soils, sediments, and other particulate matter precludes leaching to any extent. The soil-water partition coefficients, K_d, range from 1 to 88 ml/g, indicating low mobility and significant sorption.

The U. S. Geological Survey and others who monitor broadly for pesticides would be very unlikely to look for a chemical that has less than 200 pounds of usage in a year; antimycin A is not included in USGS's National Water Quality Assessment (NAWQA) program's data warehouse (http://water.usgs.gov/nawqa/data). Likewise, no antimycin A sampling data were available at California's Department of Pesticide Regulation (http://www.cdpr.ca.gov/docs/sw/surfcont.htm). Because of the lack of analytical methods for antimycin A, even targeted monitoring data from studies where antimycin was used has not been located and apparently has not been gathered.

7.5.2 Mobility of antimycin A and considerations for use in fractured basaltic areas.

The geology of eastern Washington has large expanses of fractured basalt substrate similar to volcanic areas of the Pacific Northwest, California and the Great Basin. Specific concerns have been raised about the potential migration of rotenone through the fractured basalts of the Columbia plateau, and these concerns could be raised about antimycin A also. Much of the Pacific Northwest has a highly volcanic history. Numerous layers of basalt flows, individually averaging about 100 feet thick, and collectively up to 15,000 feet thick, underlay the surface. As the lava flows cool, they tend to shrink, resulting in cracks or fissures through which liquids may permeate. Subsequent folding and faulting can also lead to openings in the layers. The tops and bottoms of these layers are particularly permeable because of fractures, vesicles and rubble zones. Unconsolidated, sedimentary soils between basalt layers may be even more permeable (USGS, 1994). At the same time, unfractured basalt layers are not permeable, and water would move laterally across these layers rather than vertically through them.

The potential movement of chemicals through fractured basaltic rocks and associated soils has become an issue in Washington as a result of studies at the Hanford site near Yakima, where radiologically and chemically contaminated water plumes are approaching the Columbia River (Williams, et al, 2000). Extensive studies by Williams, et al. (2000) and Spane and coworkers (Spane and Raymond, 1993; Spane and Vermeul, 1994; Spane and Webber, 1995**;** Spane, et al., 2001) have shown some of the Hanford aquifers are connected, while others are not, and lateral movement is as likely, or more likely than vertical movement. These studies have also demonstrated that the hydrological characteristics of such basaltic soils vary significantly. Understanding the potential movement of substances in the ground water requires a detailed analysis of an individual site, and the amount of research done to characterize the Hanford site is highly unusual.

Because the potential exists for movement through fractured basaltic soils, and because there is insufficient characterization of the hydrology for sites other than Hanford, indirect means are necessary to analyze the potential movement of antimycin A into groundwater in this geological environment. Two general aspects are important: the availability of antimycin A and the nature of the treated lake or stream and its underlying features.

As noted above (section 7.5.1), although data are limited, antimycin A is not considered mobile through soils, based upon its physical and chemical characteristics. The evidence of mobility of water soluble chemicals in the Hanford area does not apply to fairly insoluble chemicals such as antimycin A. The characterization of the geological environment of Hanford is indicative that a potential concern should be analyzed, but the situation is confounded by the wide variation in soil profiles and underlying structure in differing localities, even in close proximity.

The first consideration is related to the use of antimycin A as a piscicide. The application sites may include shallow lakes and ponds. Applications of antimycin A in streams and rivers may be more likely, but the material would move down the stream rapidly enough to have little opportunity to get into sediments. Antimycin A is not very persistent and would not be available to adsorb to sediments for very long, even in lentic waters.

To enter the fractured basaltic geologic system, antimycin A would have to persist long enough to move through the lake bed into the fractured basalt area. Once it entered the fractured basalt area, it could move either laterally or vertically through openings, fissures and cracks in the rocks. However, the potential for that movement is expected to be zero because of adsorption to sediments at the lake bottom and the immobility of antimycin A.

Lake bottoms are not simply underwater soils. Lakes that have fish also will have some level of algae and aquatic macrophytes. Decaying plant material and waste materials from aquatic animals accumulate over time and most go to the bottom of the lake creating a lake sediment that is typically rich in organic material. Even a thin sediment layer would create a barrier for antimycin A movement since it adsorbs to particulate matter and lacks mobility. This factor alone negates any movement into ground water, even in fractured basaltic areas.

Eastern Washington does, however, now have another feature that would further prevent movement through lake bottoms. In 1980, Mt St. Helens erupted and spread 540 million tons of ash over a 22,000 square mile area, covering nearly all of eastern Washington, except along parts of the Canadian border. Ash was 4-5 inches deep in Yakima and ½ inch deep in Spokane (Wikipedia entry written by Lyn Topinka, USGS, accessed online at http;//en.wikipedia.org/wiki/1980_eruption_of_Mount_St._Helens, May 16, 2007). The coarser particles that fell nearer Mount St. Helens, such as those in Yakima, would not adsorb rotenone as

much as the finer particles that traveled further, such as those in Spokane. However, the larger quantity in Yakima would substitute for the finer particles. A study in Lake Williams, near Cheney, Washington showed that ash layer was suspended for several months at the water-sediment interface before breaking up and sinking into uncompacted sediments (Anderson, et al., 1984). Presumably, a similar event would have occurred at lakes throughout eastern Washington. The fine nature of the ash, or the larger quantity of a coarser ash, either as a layer or in the uncompacted sediments, would adsorb antimycin A to the extent that none would be expected to permeate the sedimentary layer and move into the underlying strata.

Even with the limited data available for antimycin A the likelihood that it would move into groundwater through a lake bottom is negligible at most.

8. Risk Assessment and Characterization for Ecological Effects

Risk characterization is the integration of exposure and effects characterizations. From an ecological perspective, there is no risk without a combination of both toxicity and exposure. Even a relatively benign or nontoxic substance can be a risk if there is sufficiently high exposure, and even the most toxic substances are not a risk if there is no exposure. In this context, risk is a measure of the actual effects that may occur in those environments where a stressor reaches an ecological receptor in sufficient quantity. The variation in the amount and compartmentalization of a stressor and the differential sensitivity of receptors of different species, life stages, location, health, and other factors combine to result in uncertainties. There are never enough data to eliminate all uncertainties, although large quantities of data may reduce the uncertainties to levels where conclusions about risk may be predicted within certain limits.

With fewer data, more assumptions are required to assess risk. USEPA requirements, for example, include a good breadth of data to address a wide variety of risk factors. However, their data requirements do not provide a great deal of depth of information, and numerous assumptions need to be made to assess risk. From the basic toxicity requirements, for example, all avian risk projections are based upon two bird species, and likewise, all fish risk projections are based upon data for two fish species. An assumption is necessary that these birds and fish are representative of all birds and fish, or at least that they can be used as a basis for modeling for all birds and fish. Similarly, a model for an estimated environmental exposure is typically based on one or a few sites for a given a use, with the assumption that those sites used are representative of all sites.

Benchmarks are useful in this context. Based on comparable data for large numbers of chemical substances, one can look at a quantitative combination of effects and exposure, such as a risk quotient. For example, the risk quotients for a new chemical can be determined and then compared with benchmark chemicals where there is sufficient information under actual use conditions to have a reasonably good idea of what will happen. A risk quotient (RQ) is derived by dividing the environmental concentration, usually the estimated environmental concentration (EEC), of a chemical by the toxicity value, such as an LC_{50} or a no observed effect concentration (NOEC). A Level of Concern (LOC) is established by policy to achieve certain results, such as protection of populations or protection of individuals, and the RQ is compared with the LOC (Table 8.1). This is considered a "deterministic" approach, and is normally the method used unless there are extensive data available for a more refined "probabilistic assessment." For antimycin A, both EPA and this assessment use a deterministic approach.

Table 8.1. Risk presumptions used by USEPA

Risk presumption[1]	RQ[2]	LOC[3]
Acute risk - aquatic & terrestrial	EEC/LC_{50} or LD_{50}/ft^2	0.5
Acute restricted use - aquatic	EEC/LC_{50} or LD_{50}/ft^2	0.1
Acute restricted use - terrestrial	EEC/LC_{50} or LD_{50}/ft^2	0.2
Acute endangered species risk - aquatic	EEC/LC_{50} or LD_{50}/ft^2	0.05
Acute endangered species risk - terrestrial	EEC/LC_{50} or LD_{50}/ft^2	0.1
Chronic risk - aquatic & terrestrial	EEC/NOEC	1

[1]Acute risk at this level relates to effects on populations of non-target organisms
Acute restricted use relates to classification of a pesticide to be used only by certified applicators
Acute endangered species relates to effects on individuals of a T&E species
[2]EEC= estimated environmental concentration; NOEC= no observed effect concentration
The EC_{50} may substitute for the LC_{50}, especially with aquatic invertebrates
[3]LOC = Level of Concern established by US EPA as a basis for regulatory concern. Specific numbers are derived from historical information and theoretical models (Urban and Cook, 1986).

Antimycin A is somewhat unusual. The very nature of its use as a piscicide requires a "field" assessment after use to determine when rehabilitation of a water body can proceed to the next stage. That is, it is not effective to restock a treated body of water until the antimycin A has dissipated to nontoxic levels. As a result, the time it takes to reach a non-toxic environment after treatment has been determined for each treatment site, but only by bioassays to determine the toxicity of the treated water at the point which restocking the water is to begin. Additional biological monitoring may be done to assess other conditions, such as invertebrate populations in various compartments. Since each treatment site is different in the natural environment, it is difficult to extrapolate to other environments. The best field-level predictions of risk, or indeed, even efficacy, seem most likely when a project is using antimycin A in a water body that has been treated before. In the case of antimycin A, which has not been used by WDFW, any extrapolations on effects must come from antimycin A use outside of Washington state, and the uncertainties in such extrapolations can be large from site to site, probably more for non-target effects than effects on target fish. The SOP being developed should result in more consistency in antimycin A use, and would eventually reduce the level of uncertainty.

One principle of toxicology is that within the limits of genetic variability, with some consideration of factors like life stage or health, toxicity does not change for a species. What may change the risk is variation in exposure and bioavailability. On that basis, risk characterization is much more a function of environmental exposure than ecotoxicological effects. As noted, the SOP under development should result in more consistent applications and therefore environmental exposures

8.1 Direct Effects

8.1.1 Fish

Consistent with the labeled use of antimycin A as a piscicide, it is not only expected that fish will be killed from labeled use, it is intended that fish will be killed. There is a moderately large body of fish toxicity data for antimycin A, much of which was originally developed to assess efficacy and the comparative effects to different species or under different conditions (Tables 6.1, 6.2 and

6.3). The results show that the most sensitive species among those tested is the paddlefish, but for lack of sufficient raw data, EPA used the coho salmon as the most sensitive species to set its regulatory criteria. The toxicity of antimycin to sensitive fish species (coho salmon LC_{50}=0.009 µg/L) is extreme; very few pesticides have fish LC_{50} values lower than 1 µg/L and antimycin A toxicity is two orders of magnitude lower than that for many species. However, there is enough variation in species sensitivities for the labels to state that short nose gar, bowfin, goldfish, and catfish are relatively insensitive. LC_{50} values for these species are generally above 1 µg/L, which accounts for the use of antimycin A to control other fish in catfish aquaculture. Fish, apparently of all species, are also more sensitive at higher temperatures and lower pH values.

The use of antimycin A is recommended for "running water, streams, and shallow waters" (Fintrol label). It does not repel fish and therefore, barrier treatments are unnecessary when used in lentic waters with inflowing streams. Use in lentic waters with outflowing streams or use directly in streams would pose the highest risks to non-target fish. Antimycin A treatments can be used in conjunction with detoxification by potassium permanganate in lotic waters. The antimycin A RED requires that deactivation with potassium permanganate be done for any streams treated or flowing out of treated ponds or lakes, to the point at which antimycin A is no longer found at the detection limit of 0.015 µg/L (USEPA, 2007). There is evidence that antimycin A degrades very rapidly in high gradient streams where the water is highly oxygenated (Moore et al., 2005); detoxification may not be necessary in such streams, depending upon the distance and elevation drop between the treatment area and the sensitive downstream areas. However, natural degradation will no longer be allowed in such streams unless the area in which this degradation occurs is defined as part of the treatment area.

8.1.2 Other aquatic biota

Antimycin A can be highly toxic to aquatic invertebrates (Tables 6.4 and 6.5). EPA based its determination of very high risk for aquatic invertebrates on the most sensitive species, *Gammarus fasciatus*, which had an EC_{50} of 0.008 µg/L. However, the toxicity is quite variable, and for many tested invertebrates, the EC_{50} or LC_{50} values are greater than 5 µg/L.

Data are limited for applications of antimycin A to lentic waters. Houf and Campbell (1977) used antimycin A formulations on sand (no longer registered) in artificial ponds to study effects on macrobenthos. They concluded that neither 20 µg/L nor 40 µg/L antimycin treatments affected either short or long term populations of dominant benthic organisms, species diversity, or insect emergence. They did notice declines after the 40 µg/L treatment, but these started to occur before treatment and were not statistically significant due to similar declines in control ponds. However, they did note that the pH in the ponds was 9.0-9.7 which would have resulted in more rapid degradation than would occur in waters with lower pH.

Studies following application of antimycin A to lotic waters have typically found that the effects on macroinvertebrate populations are limited both in scope and duration (Pfeifer et al., 2001). Moore et al. (2005) found that after a stream treatment with 8 µg/L of antimycin A, there was an immediate reduction of 18-25% of insect taxa, with mayflies being completely eliminated. Four months after treatment, the initial acute effects had vanished, and after one year, there was no statistically significant difference with pre-treatment surveys. No dead crayfish were observed; numerous live crayfish were observed feeding on trout carcasses. Dinger and Marks (2006) found an 80% reduction in stream invertebrates following treatment with100 µg/L of antimycin A; invertebrate densities in riffle areas were particularly impacted, and those in pools were also affected. After 5 months the invertebrates "mostly rebounded." At a second site treated with 54 µg/L of antimycin A, there was significant drift of dead insects, but there was no short-term

reduction in density of invertebrates associated with pools and riffles. However, overall species diversity was still affected after 4 months. Several taxa were apparently extirpated in each treatment and had not re-appeared 4-5 months later at the end of observations.

Minckley and Mihalick (1981) also found short term effects with subsequent recovery in Ord Creek, Arizona. At 10 µg/L of antimycin A, certain mayflies, stoneflies, dipterans, and trichopterans were killed immediately and drifted downstream. They noted the absence of six species after three years, but attributed it to flooding or sampling errors rather than treatment, and concluded that there were no long term impacts on macroinvertebrates.

Cerreto (2004) found that invertebrates in two mountain streams in Wyoming treated with 10 µg/L of antimycin were not significantly different in abundance from control streams. However, the power of their test to detect effects was low. They also caged caddisflies in one creek and mayflies in the other creek and found 96% and 82% survival, respectively, which was very similar to survival in untreated (control) creeks.

Even when antimycin A is applied at high concentrations to streams, long term effects are minimal (Marks and Dinger, 2005) or none (Jacobi and Degan, 1977).

For an untested invertebrate species of concern, however, e.g., an endangered amphipod, any exposure to antimycin A should be considered problematic until and if toxicity data can be developed on that particular species. This should apply to untested T&E molluscs as well as aquatic arthropods, despite the much lower sensitivity of mollusks, relative to the *Gammarus* data used by USEPA.

There are limited data available on the effects of antimycin A on amphibians. Grisak et al. (2006) found that two adult frog species were not significantly affected at concentrations below 10 µg/L. Tailed frogs did exhibit 15% mortality at 7.5 µg/L after 96 hours, but the authors noted that mortality at concentrations typically used for fish control (5-10 µg/L), it required 72 hours and 3 daily renewals of antimycin concentrations to cause even 5% mortality. Such prolonged concentrations would not occur in stream treatments, but Grisak et al. (2006), did not determine if an initial concentration would have a lingering effect if frogs were moved to clean water. This may be the case since fish exposed to antimycin A may not recover when placed in clean water.

Additional amphibian toxicity data for antimycin A indicate that bullfrog tadpoles and adult tiger salamanders had 100% mortality at 40 µg/L for one day and 600 µg/L for 4 days, respectively. But no mortality occurred at 80 µg/L for the salamander and 20 µg/L for the bullfrog tadpoles (Walker et al., 1964). These data indicate that effects are unlikely on adult tiger salamanders. Bullfrog tadpoles were more sensitive, as might be expected from gilled stages, but even then, there was no mortality at 20 µg/L, above the typical fish treatment concentrations, but still below the maximum 25 ppb application rate proposed in the RED. It is unclear if bullfrogs are very sensitive to pesticides. In acute oral tests, Hudson, et al., (1984) found that adult bullfrogs were very insensitive to most pesticides. This may have been due to the route of exposure or possibly due to innate insensitivity of the species. Regardless of the actual sensitivity of various amphibians, if EPA were to assess the risks to aquatic stages of amphibians, their policies would indicate the use of the coho salmon (LC_{50}=0.009 µg/L) as a surrogate for amphibians.

Moore et al. (2005) found two dead, gilled salamanders after a stream treatment with 8 µg/L of antimycin A, but did not consider that there was a negative effect on the taxon, in general.

8.1.3 Terrestrial biota

Terrestrial animals most likely to be exposed are those that feed on fish. EPA has determined that there is no risk to piscivorous birds or mammals feeding on fish killed by antimycin A, even when these fish contain the maximum level of residues that have been found following antimycin A treatments (USEPA 2006a). Risk quotients for birds and mammals are well below Levels of Concern. There is some possibility of an indirect effect on the food supply of piscivorous species. But most piscivorous species would take some fish killed by the treatments, and would not be affected due to the low residues. And these piscivorous species are typically mobile and able to find other food sources.

8.1.4 Endangered and threatened species

The potential for effects on threatened and endangered (T&E) species must be considered in any fish rehabilitation project where such species may be in the vicinity. Table 8.2 presents the federal and state listed T&E species in Washington State.

Table 8.2. Endangered and threatened species in Washington

Common name	Scientific name	Status[1]
Mammals		
Bear, grizzly	*Ursus arctos horribilis*	T
Caribou, woodland	*Rangifer tarandus caribou*	E
Deer, Columbian white-tailed	*Odocoileus virginianus leucurus*	E
Fisher	*Martes pennanti*	SE
Gopher, Mazama (western) pocket	*Thomomys mazama*	ST
Lynx, Canada	*Lynx canadensis*	T
Otter. sea	*Enhydra lutris*	SE
Rabbit, pygmy	*Brachylagus idahoensis*	E
Sea-lion, Steller	*Eumetopias jubatus*	T
Squirrel, western gray	*Sciurus griseus*	ST
Whale, humpback	*Megaptera novaeangliae*	E
Whale, Sei	*Balaenoptera borealis*	E
Whale, Fin	*Balaenoptera physalus*	E
Whale, blue	*Balaenoptera musculus*	E
Whale, black right	*Balaena glacialus*	E
Whale, Killer	*Orcinus orca*	E
Whale, sperm	*Physeter macrocephalus*	E
Wolf, gray	*Canis lupus*	E
Birds		
Albatross, short-tailed	*Phoebastria albatrus*	E
Crane, sandhill	*Grus canadensis*	SE
Eagle, bald	*Haliaeetus leucocephalus*	T
Grouse, sage	*Centrocercus urophasianus*	ST
Grouse, sharp-tailed	*Tympanuchus phasianellus*	ST
Hawk, ferruginous	*Buteo regalis*	ST
Horned lark, streaked	*Eremophila alpestris strigata*	SE
Murrelet, marbled	*Brachyramphus marmoratus marmoratus*	T
Owl, northern spotted	*Strix occidentalis caurina*	T

Table 8.2. Endangered and threatened species in Washington		
Common name	**Scientific name**	**Status**[1]
Pelican, American white	*Pelecanus erythrorhynchos*	SE
Pelican, brown	*Pelecanus occidentalis*	E
Plover, western snowy	*Charadrius alexandrinus nivosus*	T
Amphibians		
Frog, northern leopard	*Rana pipiens*	SE
Frog, Oregon spotted	*Rana pretiosa*	SE
Reptiles		
Sea turtle, green	*Chelonia mydas*	T
Sea turtle, leatherback	*Dermochelys coriacea*	E
Sea turtle, loggerhead	*Caretta caretta*	ST
Turtle, western pond	*Clemmys marmorata*	SE
Fish		
Salmon, chinook (Upper Columbia River spring run)	*Oncorhynchus tshawytscha*	E
Salmon, chinook (Snake River spring/summer run)	*Oncorhynchus tshawytscha*	T
Salmon, chinook (Lower Columbia River)	*Oncorhynchus tshawytscha*	T
Salmon, chinook (Puget Sound)	*Oncorhynchus tshawytscha*	T
Salmon, chinook (Snake River fall run)	*Oncorhynchus tshawytscha*	T
Salmon, chum (Columbia River)	*Oncorhynchus keta*	T
Salmon, chum (Hood Canal summer run)	*Oncorhynchus keta*	T
Salmon, coho (Lower Columbia R.)	*Oncorhynchus kisutch*	T
Salmon, sockeye (Snake River)	*Oncorhynchus nerka*	E
Salmon, sockeye (Ozette Lake)	*Oncorhynchus nerka*	T
Steelhead (Upper Columbia River Basin)	*Oncorhynchus mykiss*	T
Steelhead (Middle Columbia River)	*Oncorhynchus mykiss*	T
Steelhead (Snake River Basin)	*Oncorhynchus mykiss*	T
Steelhead (Upper Willamette R.)	*Oncorhynchus mykiss*	T
Steelhead (Lower Columbia R.)	*Oncorhynchus mykiss*	T
Steelhead (Puget Sound)	*Oncorhynchus mykiss*	T
Trout, bull	*Salvelinus confluentus*	T
Insects		
Butterfly, Oregon silverspot	*Speyeria zerene hippolyta*	T
Checkerspot, Taylor's	*Euphydryas editha taylori*	SE
Skipper, Mardon	*Polites mardon*	SE
Plants		
Sandwort, Marsh	*Arenaria paludicola*	E
Paintbrush, golden	*Castilleja levisecta*	T
Howellia, water	*Howellia aquatilis*	T
Lomatium, Bradshaw's	*Lomatium bradshawii*	E
Lupine, Kincaid's	*Lupinus sulphureus kincaidii*	T
Checker-mallow, Nelson's	*Sidalcea nelsoniana*	T
Checker-mallow, Wenatchee Mountains	*Sidalcea oregana calva*	E
Ladies'-tresses, Ute	*Spiranthes diluvialis*	T

[1] Status is federal status, if listed. Federally listed species accessed May 13, 2007 at http://www.fws.gov/endangered/wildlife.html#Species and http://www.nmfs.noaa.gov/pr/species/esa/. If

not listed federally, status is state status i.e., SE (state endangered) and ST (state threatened), accessed at http://wdfw.wa.gov/wlm/diversty/soc/soc.htm.

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Based upon the RQs above for birds and mammals (sections 6.3.8 and 6.3.9), there is no risk for these taxa. Because avian toxicity data are used as a surrogate taxon to determine risks to reptiles, the lack of avian risk indicates no risk to reptiles. Based upon a lack of phytotoxicity to aquatic macrophytes (section 6.3.3) and an inferred lack of phytotoxicity to terrestrial plants (section 6.3.10), there is no risk to T&E plants.

The Mardon Skipper (Potter et al., 1999), the Taylor's Checkerspot (Stinson, 2005), and the Oregon Silverspot (USFWS, 2001) are all grassland species and occur in small, isolated populations. There would be negligible opportunity for exposure to antimycin A because there are only ground applications made directly to water. These would not result in exposure to terrestrial plants that might serve as host species for these lepidopterans. It is conceivable, but highly improbable, that one of these butterflies could fly in to an aquatic area being sprayed, should treatment areas be located in the vicinity of their grassland habitat. It is unknown if antimycin A has any effect on insects.

The Oregon spotted frog and the northern leopard frog are definitely associated with aquatic habitats, but might not occur where piscicide treatments are done. The sensitivity of amphibians may be low, but this is uncertain; EPA policies regarding surrogate species for amphibians would indicate high risk for T&E amphibians.. Any use of antimycin A in the vicinity of these two species should only be done after conferring with state experts on these species.

Antimycin is likely to affect any T&E fish species that is exposed; all T&E fish in Washington State are salmonids, and salmonids are among the most sensitive of all fish species to antimycin A. The most sensitive species, coho salmon, used by USEPA (2006a) for risk assessment has an LC_{50} of 0.009 µg/L. EPA would use an endangered species LOC of 0.00045 µg/L to assess risk to T&E fish. The proposed labels would require deactivation of antimycin A used in lotic waters, but only to the limits of detectability, 0.015µg/L. Thus, not even deactivation would remove EPA concerns for T&E fish in or immediately below treatment areas. Therefore, it can be assumed that any exposure to antimycin A in or downstream of a treatment site, whether detectable or not, would trigger EPA's required Endangered Species Act section 7 consultation with the U.S. Fish and Wildlife Service or National Marine Fisheries Service. WDFW lake and stream rehabilitation projects using piscicidal products purchased with US Fish and Wildlife Service Federal Aid in Sport Fish Restoration funding, or other federal funding, undergo consultation under Section 7 of the Endangered Species Act. All projects are reviewed annually by WDFW's Fish, Wildlife and Habitat program staff for potential impacts to T&E species, as well as other fish and wildlife species of concern.

WDFW does not treat waters with threatened or endangered species. According to Washington Fish and Wildlife Commission Policy C3010, waters will not be treated in ways which would cause significant negative impacts to fish or wildlife which are state or federally listed as Threatened, Endangered, Sensitive or Candidate Species**.** An exception may be granted in the case of a biological emergency. Any treatment that would "take" a federally-listed species would require a permit from the U.S. Fish and Wildlife Service or the National Marine Fisheries Service.

Antimycin A has been used in conjunction with efforts to recover T&E fish, typically by eliminating other fish that may prey upon or compete with these T&E fish. Even these beneficial

actions will trigger the consultation requirements if the project "may affect" a T&E fish species. Currently, EPA alone is responsible for a section 7 consultation on pesticide registration, although it may request help from a state or other entities. WDFW's consultations on the use of piscicides, as described above, do not address the required consultation on the registration of antimycin A by EPA. A provision of the Section 7 regulations (50CFR402.08) allows EPA, as the action agency on pesticide registrations, to name a "designated non-federal representative" to conduct a biological assessment or an informal consultation with the FWS. If a finding is expected to result in a "may affect" determination, but that the use of antimycin A is "not likely to adversely affect" the listed species, then the consultation requirement may be completed informally at the local level. At least one state agency, California's Department of Pesticide Regulation, has been named as a designated non-federal representative by the EPA. WDFW may find it advantageous to request being similarly "designated" by EPA, especially considering the number of T&E species and their broad locations within Washington.

Not all T&E fish would be expected to be exposed to antimycin A. Salmon and steelhead migrating through major rivers, such as the Columbia and Snake rivers, and the Ozette Lake sockeye salmon, which occurs within a National Park, would have no exposure. If deactivation of antimycin A by potassium permanganate is used in conjunction with applications to streams and rivers, as will be required under proposed labeling, no exposure of T&E fish would be expected to occur above detection limits. However, as noted above, non-detectability may not be sufficient to remove potential concerns. Bioassays may be more appropriate, but might not detect sublethal effects that could affect the fish. Either non-detectability or no toxicity in bioassays could be combined with sufficient dilution below the detection/assay site to indicate that antimycin A residues would be too low to be of concern. Alternatively, the flow rates of a treated stream could be used in conjunction with the amount of time it takes for a treated stream to reach sensitive waters to determine if degradation of antimycin A is likely to have occurred before a T&E fish is exposed.

8.2 Effects on water quality

There are no direct data available to assess the effects of antimycin A applications to water quality parameters such as pH, dissolved oxygen, nitrate, nitrite, and ammonia production, and the release of phosphates. There are no phosphorous or nitrogen components of antimycin A that could be released into treated water. However, in his assessment of rotenone, Bradbury (1986) discussed the effects of a piscicide on water quality. He suggested that algal blooms following piscicide treatments may be due, in part, to the release of phosphorus from decaying fish. Bradbury further indicated that rotenone treatments would have negligible effect on dissolved oxygen, pH, temperature, alkalinity, or carbon dioxide, and it would appear that those conclusions would be valid for antimycin A also. Antimycin A is said to impart no taste or odor to water (Fintrol label).

8.3 Effects from interactions with other pesticides

No data are known on the interaction of antimycin A with other pesticides.

8.4 Effects on pristine and contaminated sites

No data are available. Presumably there would be no use in contaminated sites. Toxicity data are generated in what could be considered pristine waters and should therefore apply to pristine sites.

8.5 Indirect effects

8.5.1 From removal of fish and other aquatic biota

The loss of fish in a water body would potentially have an effect on piscivorous animals. Similarly, the loss of other aquatic biota could have an effect on predators of those biota. However, it is WDFW's intention to treat only shallow ponds and streams with antimycin A. With such small areas being treated, piscivorous birds and mammals will have enough mobility to move to nearby to non-treated areas. Since large bodies of water will not be treated with antimycin A, even birds feeding young at the time of treatment should not be affected because there would be nearby alternative food sources.

WDFW rehabilitation planning usually includes the provision that dead fish remain in the stream (rather than be removed from the water), specifically to provide nutrients for invertebrate growth following the piscicide treatment. These invertebrates provide a food base for fish that are re-stocked into the waters following the treatments. The dead fish would provide an immediate food source for crawfish, amphipods, insects and other aquatic species. However, proposed new labeling states, "The Certified Applicator or designee under his/her direct supervision should collect and bury dead fish" (Table APP-1). Another provision states, "Where practical, users should collect and bury dead fish" (Table APP-2). These statements, if adopted with the "should" language, would not preclude WDFW from leaving the dead fish as nutrients for invertebrates.

8.5.2 Potential for increased erosion and resuspension of soils and sediments resulting from effects on plants

Based upon the ground application directly to aquatic sites, there is no potential for erosion and no expectation of resuspension of soils and sediments.

8.5.3 Effects on aquatic habitats

Effects on aquatic habitats would not be expected from antimycin A, with some uncertainty, because there is no evidence that antimycin A would affect the plants or the water chemistry except for the potential for algal blooms resulting from the potential loss of zooplanktonic grazers on algae and release of phosphorus from decaying fish. The effects of rotenone apply here because indirect effects could be expected to be similar. Of the 9 rotenone-treated lakes analyzed by Bradbury (1986) only three had what were termed "major blooms" of algae, and the duration of these three "generally lasted 1-2 months." The effects of antimycin A on aquatic invertebrates at labeled application rates are considerably lower than for rotenone, and any effects that are based upon elimination of fish would be short-lived.

8.5.4 Potential effects upon agriculture

Based upon the ground application directly to aquatic sites, there is no potential for effects upon agricultural crops except as might result from use of treated water for irrigation. There are no data available to address effects on agricultural crops should treated water be used to irrigate these crops. The concentrations of antimycin A that could be in irrigation water, according to proposed labeling, would have to be below 0.015 µg/L, which should be insignificant. Streptomyces molds, from which antimycin A is extracted, are a natural component of soils, and would not be expected to have an adverse effect on agriculture.

8.5.5 Indirect effects on endangered and threatened species

Indirect effects on T&E species would be those that would affect the food or habitat of a species. Effects on food would be the most likely and piscivorous species would be the most likely ones affected. However, typical antimycin A treatment areas would be small relative to the feeding range of species that might be in the treatment areas. Only if there were wide-area treatments with antimycin A would there be any potential indirect effects on piscivorous species.

8.6 Impacts of multiple applications

Antimycin A has limited persistence in the environment. The only reason that multiple applications could occur in the same year would be if the first treatment was not effective, which in turn would imply that there was an insufficient amount of antimycin A to result in any accumulated concentrations in the water from one treatment to the next. And, if the first treatment was not effective, then there would not be any cumulative indirect impacts on organisms of significance.

8.7 Impacts on terrestrial organisms and environments

Based upon ground application directly to aquatic sites, there is negligible potential for impacts on terrestrial biota other than unlikely effects that might occur if treated water is used to irrigate crops. There are no available data to quantify any terrestrial exposures.

8.8 Impacts on wetlands other than target application sites

Except for wetlands downstream from treatment sites, there would be no exposure of antimycin A to untreated wetlands. Under the proposed label requirements, antimycin A would have to be deactivated as it left the treated water, thus precluding and exposure of downstream wetlands of any type. Under current labels, it is possible for antimycin A to reach downstream wetlands if it is not deactivated, but even this would be limited because of the low concentrations and rapid degradation.

8.9 Uncertainty analysis

There are a number of uncertainties in this analysis. The toxicity profile for antimycin A is relatively complete for fish, but not very broad for aquatic invertebrates. It is limited on aquatic macrophytes and non-existent for algae. Some anecdotal information exists on the latter two groups. Toxicity data on amphibians are limited and not well characterized. No data were located on the aquatic toxicity or effects of antimycin A degradates, although these would likely be in such low concentrations as to be of no concern.

The environmental fate profile of antimycin is very limited, and includes many estimates from physical and chemical properties, several hydrolysis studies and an aerobic metabolism study. Most other fate and transport data requirement have been waived.

There is some uncertainty on the use of potassium permanganate to deactivate antimycin A. Based upon the use of potassium permanganate to deactivate rotenone and the problems that have occasionally resulted (Finlayson et al., 2000), there is a potential that similar problems in ensuring appropriate concentrations could happen with antimycin A. This should be addressed in the forthcoming SOP for antimycin A.

There is also uncertainty on what changes may occur with the antimycin A labeling or other requirements. A 60-day public comment period for the RED began on June 13, 2007 when the RED was posted to EPA's antimycin docket; this comment period will close August 13, 2007. There may be alterations in the proposed requirements as a result of this comment period. In addition, a Standard Operating Procedure manual is required and no details are yet known about how and when this manual will be developed. As currently proposed, it will be a mandatory requirement to follow this manual.

8.10 Additional needs for information

8.10.1 Soil and sediment

At this time, data on antimycin and soils or sediments is limited to estimates. In addition, the lack of an analytical method to detect low concentrations of antimycin A expected to be found in the environment has limited both laboratory studies on chemical fate and transport and, more importantly, knowledge of antimycin A residues that might result from its use. Information on field measurements of antimycin A residues in sediment would markedly enhance an analysis of its compartmentalization and persistence.

8.10.2 Water

Standard aquatic field dissipation studies are important for understanding the persistence in the water when sediments are present. Some data are available, but they are limited.

8.10.3 Plants

Toxicity data on aquatic macrophytes are limited. Such data would be useful for assessing the impacts, or lack thereof, of antimycin A. More importantly, they would provide a basis for assessing the risks to T&E plant species, such as the Water Howellia. Such risks may not be expected, but actual data would be valuable to document those risks. Terrestrial plant toxicity data are absent, but the likelihood of exposure of terrestrial plants is very low.

8.10.4 Acute toxicity studies

The acute toxicity data base for antimycin is weak for terrestrial organisms. There is a reasonable acute toxicity data base for fish, but testing on a wider variety of aquatic invertebrates would help to better characterize the impacts on this taxa and those organisms that rely on them for food.

8.10.5 Chronic toxicity studies

Chronic aquatic toxicity data are essentially non-existent for antimycin A. However, EPA has indicated that antimycin A will not persist in the environment. Antimycin A applied to flowing waters would eventually be flushed through the system and would be diluted by untreated tributaries. Since only one treatment per year is expected, the potential for chronic ecological exposure is low, and outflowing water will have to be deactivated under the proposed labeling requirements. In lieu of any chronic toxicity data requirements, EPA uses the most conservative models to assess chronic risk. Additional data would be useful if there are reasons to refine the chronic effects profile beyond what is done through the use of these conservative assumptions.

8.11 Mitigation measures

The requirement for deactivating antimycin A in streams or waters flowing out of lakes, as required in the proposed labeling, is the most thorough mitigation measure that could be applied

to antimycin A for non-target species in general. For T&E species considerations, thorough site characterization, including documenting the locations for these species, would be important.

8.12 Conclusions and recommendations

Based upon this analysis and the proposed labeling and other requirements in the U. S. Environmental Protection Agency's Reregistration Eligibility Decision document, the potential adverse environmental effects of antimycin A, if it were to be used as a piscicide in Washington State, should be limited to the sites specifically treated and the target fish species. Some effects may occur on aquatic macroinvertebrates, but based on considerable experience, any such effects are likely to be brief if application rates are held to 10 µg/L, and should last for only a few weeks if applications are made at somewhat higher rates.

9. Human Health Effects

9.1 Objective and approach

There are very few data to address the health effects of antimycin. There are no data requirements in the RED (USEPA, 2007), primarily because of the very small amount used and the aquatic use. Rather, EPA has taken a very conservative approach which is intended to protect human health by avoiding exposure. Registrants may submit exposure or toxicity data that may result in the removal or reduction of labeled restrictions or required mitigations, but they are not required to do so.

9.2 Toxicity information and sources

Many health effects data, other than basic acute toxicity data, are lacking for antimycin A. Based upon there being only an aquatic use and the very small amount used per year (less than 200 pounds), EPA has waived the missing data. Instead, there will be label revisions that are expected to preclude human exposure and thus, effects on the human population (see section 3.4 and Appendix 1).

9.2.1 Acute

9.2.1.1 Oral

The acute oral toxicity of technical grade antimycin A has not been quantitatively determined. EPA indicated that there was "limited" information indicating that a Toxicity Category I classification is warranted. Test data on the Fintrol formulated product is presented in Table 9.1.

Table 9.1. Acute Oral Toxicity of Antimycin A to Rats from USEPA (2006d)

Formulation	% a.i.	Toxicity	MRID
Fintrol	23%	Males: LD_{50}=286 mg a.i./kg Females: LD_{50}=361 mg a.i./kg Combined : LD_{50}=316	00145496

9.2.1.2 Dermal

One dermal toxicity test on the 23% a.i. Fintrol Concentrate indicated that there is no dermal toxicity of this formulation (table 9.2).

9.2.1.3 Inhalation

Inhalation data exist for both the technical antimycin A and the 23% a.i Fintrol Concentrate (table 9.2). The results warranted a Toxicity Category rating of II ("warning") for the technical grade, but only a toxicity category IV rating for the Fintrol Concentrate (table 9.3).

9.2.1.4 Irritation and sensitization

The data presented in the health effects analysis (USEPA, 2006d) are not clear on whether there was one eye irritation test reported in two parts or two different tests (table 9.2). It is clear that the eye irritation for the Fintrol Concentrate formulation warranted a Toxicity Category of II ("warning") (Table 9.3). Additional test data indicate no dermal irritation, but it is unclear whether this is for the technical antimycin A or the formulated Fintrol Concentrate.

Table 9.2. Acute Toxicity, other than oral, of Antimycin A from USEPA (2006e)

Study Type	% Antimycin A	Toxicity	MRID or other EPA Reference
Acute Dermal – rat	23%	LD_{50}>5000 mg/kg	46752604
Acute Inhalation – rat	tech	LC_{50}<0.166 mg/L combined	1993 EPA review (D189202)
Acute inhalation - rat	23%	>2.59 mg/L	46762605
Acute Eye Irritation – rabbit	??	Irritation resolved within 48 hrs for a 0.5% solution Opacity – unwashed – some opacity remained at 24 hr; washed – only looked at 1 hr and had 1/3 opaque and 2/3 translucent	1993 EPA review D189202
Acute dermal irritation – rabbit	not reported	Not a dermal irritant	46762602

9.2.1.5 FIFRA Toxicity Categories for various exposure routes

EPA assigns Toxicity Categories for various exposure routes to humans (Table 9.3). Each category is to be designated by a "signal word" that appears on the label. For toxicity category I, in addition to the signal word of "danger", the label must also use the word "poison" and have a skull and crossbones displayed near the word "poison."

Table 9.3 EPA Acute Toxicity Categories for Antimycin A from EPA (2006d)

Route of Exposure	Toxicity Category	Signal Word(s)
Oral	I (II for Fintrol)	**Danger, Poison**
Dermal	IV (for Fintrol)	**Caution**
Inhalation	II (IV for Fintrol)	**Warning**
Dermal irritation	Not indicated	**Caution**
Eye irritation	II (for Fintrol)	**Caution**

9.2.2 Pharmacokinetics

No data are available. Antimycin A acts by uncoupling oxidative phosphorylation by blocking the electron transport pathway to Complex III within the mitochondria.

9.2.3 Subchronic

In a 90-day subchronic rat study with the 23% a.i. Fintrol Concentrate formulation, the LOAEL was determined to be 0.5 mg/Kg/day based on diarrhea and soft stools. A NOAEL was not established. The increased incidence of diarrhea and soft stool is most likely due to the effect of antimycin on intestinal flora. No other adverse effects were reported that were considered to be toxicologically significant

9.2.4 Chronic

No data are available.

9.2.5 Reproductive and developmental toxicity

No data are available.

9.2.6 Mutagenicity and carcinogenicity

No data are available.

9.2.7 Epidemiology

No data are available.

9.2.8 Incident reports

No incidents have apparently been reported for antimycin A; none were noted in the RED (USEPA, 2007).

9.3 Exposure assessment

9.3.1 Exposure routes

9.3.1.1 Swimming

According to the current antimycin A label, swimming is allowed in treated water only after a bioassay with a sensitive fish shows no toxicity for 48 hours. The proposed label would require analytical measurements of antimycin A to be below the 0.015 µg/L detection limit before swimming is allowed in treated water. Antimycin A is, according to the label, for use in shallow waters and streams. Neither of these sites would be particularly desirable swimming areas. As with many of the proposed labeling requirements, the purpose here is to preclude human exposure to risks that are not fully understood, and not because of a specific known risk. If new data were generated to address exposure by swimming, the proposed label requirements regarding this kind of exposure might be reduced or possibly even eliminated.

9.3.1.2 Drinking water

As with exposure by swimming, the current antimycin A label allows treated water to be used for drinking by humans or livestock only after a bioassay with a sensitive fish shows no toxicity for

48 hours. The proposed label would require analytical measurements of antimycin A to be below the 0.015 µg/L detection limit before treated waters could be used for drinking. As with many of the proposed labeling requirements, the purpose here is to preclude human exposure to risks that are not fully understood, and not because of a specific known risk. If new data were generated to address drinking water exposure, the proposed label requirements regarding this kind of exposure might be reduced.

9.3.1.3 Applicator exposure

The current label requires only the use of gloves and goggles for applicators. Proposed requirements for applicators specify protective eyewear, chemical resistant gloves, long-sleeved shirt, long pants, and shoes and socks. In addition, applicators using handheld equipment or handheld nozzles must wear a dust/mist respirator and coveralls. Mixer/loaders and others handling the concentrate must also wear a dust/mist respirator and a chemical-resistant apron. Wearing of contact lenses while handling or applying the product is to be prohibited. As noted above, the EPA approach for antimycin A is to conservatively preclude exposure, given the lack of applicable toxicity data.

9.3.1.4 Other

Current labels state that fish killed by treatments "should" not be consumed. Proposed labeling will specifically prohibit consumption of dead fish from treated waters.

10. Risk Assessment and Characterization for Health Effects

10.1 Drinking water, irrigation water, and swimming

Under current label provisions, human exposure through drinking water, swimming, and use of treated water on crops all require a bioassay with sensitive fish before drinking, swimming, or irrigating. Sensitive fish LC_{50}s are typically under 0.2 µg/L for bluegill and 0.1 µg/L for rainbow trout. If no individuals of a sensitive fish population die in a bioassay, then it seems likely that antimycin A concentrations would be below 0.02 and 0.01 µg/L, respectively, for bluegill and rainbow trout. This is based upon a number of assumptions spelled out in Urban and Cook (1986), and has considerable uncertainty, especially regarding the slope of the concentration-response curve. But it does suggest a reasonable level of protection. The proposed labeling requirements state that a chemical analysis must be below the detection levels of 0.015 µg/L before any of these activities can occur. This is not much different than would be likely from a bioassay approach, but it is more quantifiable and does not depend upon the nature of the test fish.

10.2 Fish consumption

Under current labels, dead fish from treated waters "should" not be consumed. Assuming that residues in dead fish would be 172 µg antimycin/Kg of fish body weight, as determined in the EFED chapter (USEPA, 2006a), then ingestion of a Kilogram of fish would provide a dose of 172 µg of antimycin, which would be 8.6 µg /Kg for a 70 Kg person. With a mammalian (rat) LD_{50} of 316 mg/Kg for males and females combined, and a safety factor of 1000, levels of concern would be for ingestion of 316 µg/Kg for the formulated product. This would be equivalent to an LD_{50} of 72.7 µg/Kg for 100% active ingredient, or about 8 times higher than would occur from eating the dead fish. It would not appear that this level of consumption would be an acute risk to

human health. However, the proposed labeling would prohibit eating fish from treated waters. With this provision, there would be no risk.

10.3 Exposure during applications

The data to quantify exposure to applicators and other handlers of antimycin A are meager. EPA did not attempt to quantify occupational exposure (USEPA, 2006d) under either current labels or the proposed labels. The proposed labeling should be more than sufficient to preclude exposure at levels of concern. For applicators who wore only the goggles and gloves specified on old labels, some occupational exposure could occur through dermal contact and inhalation. Toxicity via these routes is relatively low for the Fintrol Concentrate product, but inhalation toxicity is more pronounced with technical antimycin A. Given the weakness of the toxicity data base, the requirement for additional Personal Protective Equipment is appropriate.

10.4 Chronic exposure

With typically only one application per year, and relatively rapid degradation of antimycin A, even if not detoxified, the potential for chronic exposure and effects to humans is very limited, if it exists at all.

10.5 Uncertainties

The uncertainties associated with antimycin A and health effects are largely ones of a very meager data base with respect to both toxicity and exposure. They are compounded by a former lack of analytical methods to detect antimycin A in the environment. As a result no quantifiable risk assessment can be developed, and human health is protected by being very conservative with regard to exposure to antimycin A, particularly occupational exposure. Thus, the uncertainties do not need to be resolved to ensure that health is protected. However, there may be room for considerably fewer or less pronounced protective measure if these uncertainties are addressed by data in the future. USEPA (2007) has made it clear that additional data may be submitted, even if it is not required, to address/rebut some of the very conservative assumptions.

10.6 Conclusions

Under current labels, effects on human health as a result of occupational exposure or exposure to treated water used for drinking, irrigation, or swimming, appear to be rather low as a result of the low application rates relative to the toxicity of antimycin A. However, fully defensible conclusions cannot be made on the basis of the very meager data. Suffice it to say that the history of use, the low annual usage, and the limited exposure all work to limit the risk of antimycin A to humans.

EPA is attempting to preclude essentially all human exposure with the proposed label requirements. As a result, if the proposed labeling requirements become final, there should be no effect on human health. Even if there were relaxations of these proposed label changes, the RED indicates that these would have to be based upon submission of valid data that would address certain features of exposure and/or toxicity, and presumably any reductions in the requirements would consequently still be protective of human health.

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Appendix 1

Table APP-1. Risk Mitigation Measures for Antimycin A from RED (USEPA, 2007)	
Risk of Concern	**Mitigation Measures**
Exposure from consuming treated water may pose risks of concern	• Flowing water (including outflow from standing water such as lakes and aquaculture ponds) from treatment areas must be deactivated with potassium permanganate. • Drinking water intakes within the treatment area must be closed during treatment and until monitoring samples demonstrate antimycin A levels are below the limit of detection (0.015 ppb).
Exposure from consuming treated fish may pose risks of concern	• Through posting and access area closures, the Certified Applicator or designee under his/her direct supervision must prohibit consumption of dead fish taken from treatment areas. • The registrant must amend labels to specify maximum treatment concentrations of 10 ppb for use as a 'selective kill' in aquaculture. • When antimycin A is applied as a selective kill in aquaculture, the Certified Applicator must inform the owner/operator of the aquaculture site being treated that surviving fish must not be harvested for food or feed for a minimum of 12 months after treatment.[1] • When antimycin A is applied as a complete kill in aquaculture, the Certified Applicator must inform the owner/operator of the aquaculture site being treated that the water body must not be restocked for a minimum of 7 days after treatment.
Exposure from performing recreational activities in treated water may pose risks of concern	• Through posting and access area closures, the Certified Applicator or designee under his/her direct supervision must prohibit recreational access (e.g., wading, swimming, boating and fishing) to the treatment area during treatment and for 7 days after treatment.

Occupational exposure may pose risks of concern	• The registrant must amend labels to specify maximum treatment concentrations of 25 parts per billion (ppb). • The registrant must amend labels to require antimycin A applications to be supervised by an on-site Certified Applicator. The on-site Certified Applicator should attend a certification program for piscicide applications. • The registrant must amend labels to require all mixers/loaders and others exposed to the concentrate (e.g., through cleaning equipment) to wear long-sleeved shirt and long pants, chemical-resistant gloves, shoes plus socks, protective eyewear, a dust/mist respirator, and a chemical-resistant apron. • The registrant must amend labels to require all applicators and other handlers to wear long-sleeved shirt and long pants, chemical-resistant gloves, shoes plus socks, and protective eyewear. • In addition, applicators using handheld equipment or handheld nozzles must wear a dust/mist respirator and coveralls. • The registrant must amend labels to prohibit handlers from wearing contact lenses while handling this product.
Ecological risk quotients (RQ) for non-target species exceed OPP's level of concern	• The registrant must amend labels to prohibit antimycin A use in estuarine/marine environments. • Through deactivation with potassium permanganate, the Certified Applicator or designee under his/her direct supervision must ensure that antimycin A will not affect areas beyond the treatment area. • The Certified Applicator or designee under his/her direct supervision should collect and bury dead fish.

[1] The registrant may request that EPA remove or reduce this restriction upon submission of acceptable toxicity and exposure studies that demonstrate risk from consuming fish is below OPP's level of concern

Table APP-2. Label Changes Summary Table for Antimycin A End-Use Products from RED (USEPA, 2007)	
Description	**Amended Labeling Language for End-Use Products**
RUP	"Restricted Use Pesticide" "Due to toxicity to fish and other aquatic organisms and the need for specialized applicator training." "For retail sale to and use by only Certified Applicators or persons under their direct supervision and only for those uses covered by the Certified Applicator's certification."
SOP Manual	"THIS PRODUCT MUST BE ACCOMPANIED BY AN EPA-APPROVED PRODUCT LABEL AND THE EPA-APPROVED 'ANTIMYCIN A STANDARD OPERATING PROCEDURES MANUAL.' THE ANTIMYCIN A STANDARD OPERATING PROCEDURES (SOP) MANUAL IS LABELING. READ AND UNDERSTAND THE ENTIRE LABELING AND SOP MANUAL PRIOR TO USE. ALL PARTS OF THE LABELING AND SOP MANUAL ARE EQUALLY IMPORTANT FOR SAFE AND EFFECTIVE USE OF THIS PRODUCT."
PPE Requirements Established by the RED[1] for all Formulations	"Personal Protective Equipment (PPE)" "Some materials that are chemical-resistant to this product are" [EUP registrant, insert correct chemical-resistant material]. "If you want more options, follow the instructions for category" [EUP registrant, insert A, B, C, D, E, F, G, or H] "on an EPA chemical-resistance category selection chart." "All mixers/loaders and others exposed to the concentrate through cleaning equipment or spills must wear: * long-sleeved shirt and long pants, * chemical-resistant gloves, * shoes plus socks, * protective eyewear, * a dust/mist filtering respirator (MSHA/NIOSH approval number prefix TC-21C), or a NIOSH approved respirator with any N, R, P, or HE filter, and * a chemical-resistant apron." "All applicators and other handlers must wear: * long-sleeved shirt and long pants, * chemical-resistant gloves, * shoes plus socks, and * protective eyewear." "In addition, applicators using handheld equipment or handheld nozzles must wear: * a dust/mist filtering respirator (MSHA/NIOSH approval number prefix TC-21C), or a NIOSH approved respirator with any N, R, P, or HE filter, and * coveralls Exception: if the applicator is exposed to splashing water or walking in water that is being treated, chest waders must be worn instead of coveralls."
User Safety	"Do not wear contact lenses while handling this product. Ocular

Requirements	contact with this product can melt a contact lens onto the eye." "Follow manufacturer's instructions for cleaning/maintaining PPE. If no such instructions for washables exist, use detergent and hot water. Keep and wash PPE separately from other laundry." "Discard clothing and other absorbent materials that have been drenched or heavily contaminated with this product's concentrate. Do not reuse them."
User Safety Recommendations	"User Safety Recommendations" "Certified Applicators applying or supervising the application of this product should attend a training program for piscicide applications." "Users should wash hands before eating, drinking, chewing gum, using tobacco, or using the toilet." "Users should remove clothing/PPE immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing." "Users should remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing."
Environmental Hazards	"Environmental Hazards" "This product is extremely toxic to fish and other aquatic organisms." "Do not contaminate water by cleaning of equipment or disposal of equipment wash waters."
Personal Protective Equipment When Re-entering Treated Areas	"Re-entering the Treatment Area" "For the first 7 days after treatment, handlers re-entering treated water must wear the following PPE: * Chest waders over long-sleeved shirt, long pants, * chemical-resistant gloves, * shoes plus socks, and * protective eyewear."
Complete and Selective kills	"Complete and Selective Kills" "This product may be used to achieve a 'complete kill' or a 'selective kill.' Complete kills are intended to eliminate all fish in the treatment area whereas selective kills, used only in aquaculture, are intended to eliminate or reduce the number of only certain (more vulnerable) species. Detailed instructions for conducting complete and selective kills are presented in the Antimycin A SOP Manual."
General Application Restrictions for all Formulations	"The Certified Applicator supervising the treatment must remain on-site for the duration of the application." "The Certified Applicator supervising the treatment must not allow recreational access (e.g., wading, swimming, boating, fishing) within the treatment area while antimycin A is being applied and for 7 days after treatment. See Placarding of Treatment Areas for additional requirements and information." "For 'complete kill' use, do not apply this product in a way that will result in treatment concentrations greater than 25 parts per billion. For 'selective kill' use in aquaculture, do not apply this

	product in a way that will result in treatment concentrations greater than 10 parts per billion." "Do not apply this product in a way that will contact workers or other persons, either directly or through drift. Only protected handlers may be in the area during application." "Do not apply for with any application method or equipment not specified on this label or in the SOP." "Do not apply in a manner not specified on this label or the Antimycin A SOP Manual." "This product must not be applied to estuarine or marine environments." "Where practical, users should collect and bury dead fish."
Additional Requirements for Use in Aquaculture	"Additional Requirements for Use in Aquaculture" "When antimycin A is applied as a selective kill in aquaculture, the Certified Applicator supervising the application must inform the owner/operator of the aquaculture site being treated that surviving fish must not be harvested for food or feed for a minimum of 12 months after treatment." "When antimycin A is applied as a complete kill in aquaculture, the Certified Applicator supervising the application must inform the owner/operator of the aquaculture site being treated that the water body must not be restocked for a minimum of 7 days after treatment."
Drinking Water Notification Requirements	"Drinking Water Notification" "If drinking water intakes are present within the treatment area, prior to application, the Certified Applicator must provide notification to the party responsible for the public water supply or to individual private water users. Drinking water intakes within the treatment area must be closed during treatment and until monitoring samples demonstrate that antimycin A levels are below the limit of detection (0.015 ppb)." "Detailed instructions for public involvement, notifications, and monitoring are presented in the Antimycin A SOP Manual."
Notification Requirements for all applications except aquaculture applications	"Placarding of Treatment Areas, Except Aquaculture Applications" "The Certified Applicator in charge of the application (or someone under his/her supervision) must placard all access areas to the treatment area. Detailed instructions for placarding are presented in the Antimycin A SOP Manual. At a minimum, placards must be placed every 250 feet (including the shoreline of the treated area and up to 250 feet of shoreline past the application site to include immediate public access points) and contain the following information:" "NOTICE: AREA CLOSURE" * Skull and crossbones symbol * "DANGER/PELIGRO" * "DO NOT ENTER/NO ENTRE: Pesticide Application" * The name of the product applied * The agency or entity performing the application * The purpose of the application

	* The start date and time of application * The end date and time of application * The duration of the area closure * "Recreational access (e.g., wading, swimming, boating, fishing) within the treatment area is prohibited while antimycin A is being applied and for 7 days after treatment." * "In standing water treatment areas (non-flowing water), do not swim or wade in treated water for a minimum of 7 days after the last application." * "Do not consume dead fish from treated water." * The name, address, and telephone number of the Certified Applicator in charge of the application "Signs must remain legible during the entire posting period and must be removed no earlier than 7 days after treatment and no later than 14 days after treatment."
Deactivation with Potassium Permanganate	"Deactivation with Potassium Permanganate" "Flowing water (including outflow from standing water) must be deactivated with potassium permanganate to prevent exposure beyond the defined treatment area. Detailed instructions for deactivation with potassium permanganate are presented in the Antimycin A SOP Manual."

[1] PPE that is established on the basis of Acute Toxicity of the end-use product must be compared to the active ingredient PPE in this document. The more protective PPE must be placed in the product labeling. For guidance on which PPE is considered more protective, see PR Notice 93-7.